CHEMICAL INVESTIGATIONS IN THE HEMICELLULOSE GROUP, WITH SPECIAL REFERENCE TO THE GALACTAN COMPOUNDS.

-By-

Else Ramstad, Cand.real. (Oslo).

A thesis presented for the degree of Doctor of Philosophy.

University of Edinburgh. May 1956.

CONTENTS

INTRODUCTION

I N T R O D U C T I O N.

Woody tissue contains carbohydrates, lignin, organic extractives, and inorganic salts. Of these, the carbohydrate material is the principal constituent, being present in the amount of 70-80%. Lignin comes next in quantity (about 25%). The extraneous components of wood differ very widely in type and in amount. Some tropical woods may contain nearly 40%, while others (like yellow birch) may contain only 2-4% of total extractives.

The fundamental skeletal substance of the cell consists mainly of α-cellulose, but the cell wall is made up also from other carbohydrates. Except for cotton which is almost pure cellulose, the long cellulose bundles in plant-cells are embedded in an amorphous mass of lignin and polysaccharide material (1a).

Before the carbohydrates in wood can be investigated, interfering substances have to be removed. The lipids and other extractives are usually removed with hot alcohol and ether. Lignin is made water soluble after mild treatment with chlorine or sodium chlorite. Of the original wood remains now only the so-called holocellulose which is treated with aqueous alkali. The alkali leaves the insoluble α-cellulose and dissolves a mixture of several polysaccharides. These alkali soluble materials are called hemicelluloses, because

they once were presumed to be related to cellulose.

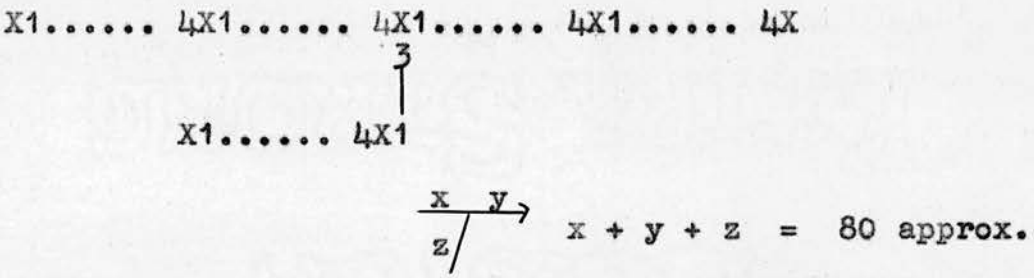
The polysaccharides in hemicelluloses consist of many different sugar residues. Some of them give strength and rigidity to the cell walls, while others appear to be concerned with metabolic processes.

The exact nature of the hemicelluloses is not yet known. Their complexity makes it difficult to separate them into individual polysaccharides. A wide variety of different molecular sizes and molecular shapes are represented, and each molecular type may occur in different degrees of polymerization. Sugars most common among the hydrolytic products are D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose, and the glycuronic acids D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and possibly D-galacturonic acid.

D-Xylose is the sugar most frequently present in hemicelluloses. Xylans are found in all lignified plant tissues and form a considerable part of the dry weight of all woods, cereal straws, seed coats and similar material. Several investigations have been carried out on xylans of different origin.

One of the main difficulties has been to obtain a pure xylan free from other polysaccharides, especially cellulose and araban. Chanda, Hirst *et al.*(1) studied xylan from Esparto grass (*Stipa tenacissima*) and by means of the

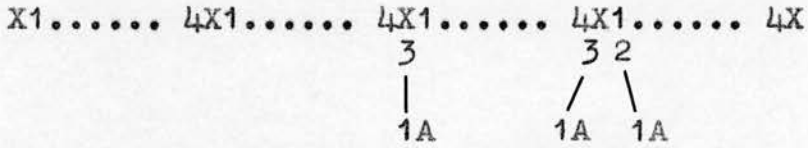
insoluble copper-complex xylan gives in alkaline copper-solution, they managed after several precipitations to get a pure xylan. After methylation and hydrolysis of the methylated product they got 2:3-di-O-methyl-D-xylose (over 90%) and 2:3:4-tri-O-methyl-D-xylose (2.5%), together with some 2-O-methyl-D-xylose. The following formula (I) was suggested for pure xylan from Esparto grass.



I.

The arabinose-rich fraction of Esparto xylan was not further investigated till after Perlin (2) had achieved some interesting results in his work on a xylan from wheat-flour. Perlin found that the arabinose content (37%) could not be altered by fractionation. Mild hydrolysis, however, removed arabinose completely, and left a true xylan. The methylated araboxylan gave on hydrolysis equal amounts of 2:3:5-tri-O-methyl-L-arabofuranose and 2:3-di-O-methyl-D-xylose, together with 2-O-methyl-D-xylose and free D-xylose.

Wheat-flour xylan has a chain of xylopyranose residues, similar to that of Esparto xylan, but with numerous single arabofuranose residues attached as sidechains (II).



X = xylopyranose.

A = arabofuranose.

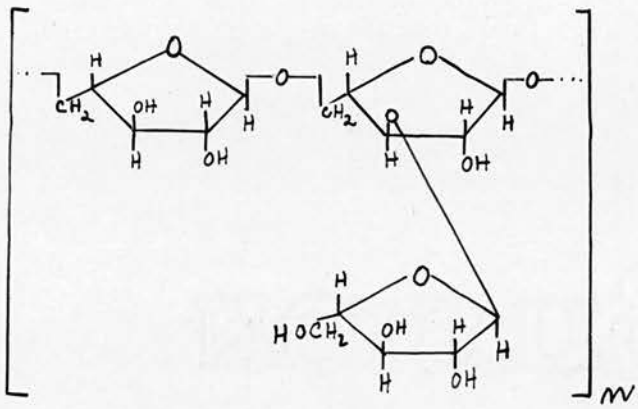
II.

When the arabinose-rich fraction of Esparto xylan was examined (3) it appeared to have a structure similar to that of wheat-flour xylan.

Though arabinose and galactose are widely distributed in the higher plants, pure arabans and two true galactans have so far only been obtained from pectic substances.

Hirst and Jones studied several arabans of which the araban isolated from the peanut (Arachis Hypogea)(4) will be mentioned. The ease of hydrolysis indicated that the polysaccharide was built up of L-arabofuranose residues. The methylated product had a specific optical rotation $[\alpha]_D^{20}$ - 180°, and yielded on methanolysis equal molar quantities of methyl 2:3:5-tri-O-methyl-L-arabofuranoside, methyl 2:3-di-O-methyl-

-L-arabinoside and methyl 2-O-methyl-L-arabinoside. After these results and the rotational data that showed the L-arabofuranoside links to have the α-configuration, the possible structure is as shown (III).



III.

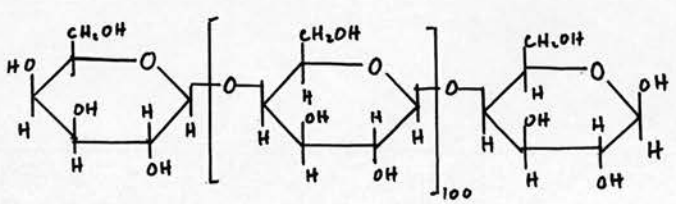
A galactan from the seeds of Strychnos nux-vomica has been studied by Andrews, Hough and Jones (4a). The polysaccharide had an optical rotation $[\alpha]_D$ + 69°. Methylation and periodate studies revealed that its structure consisted of chains of β-D-galactopyranose units, the majority being linked through C1 and C4, but probably with a small number of branching points

arising from units linked through C1, C3 and C6.

Another galactan has been obtained reasonably pure from the seeds of Lupinus albus (5). A galactan has also been reported to be present in spruce wood (6), but no methylation or other structural studies were carried out.

In admixture with the galactan from Lupinus albus (5) was araban which was removed by extraction with 70% alcohol. The galactan, which still contained some araban, was methylated and the methylaraban separated from the methylated galactan by extraction of the former from the mixture with ether.

The ether-insoluble material had a specific optical rotation $[\alpha]_D^{20}$ - 12° in methanol, which is an indication that the sugar residues are joined together by β-linkages. The methylated product was resistant to hydrolysis, the rate of which corresponded to that of a typical galactopyranoside. On methanolysis it yielded methyl 2:3:6-tri-O-methyl-D-galactoside and small amounts of methyl 2:3:4:6-tetra-O-methyl-D-galactoside. It was suggested to contain a straight chain of galactopyranose units (IV).



IV.

Fractionation studies of the methylated galactan showed, however, that extraction with ether had not removed araban completely. The methylated galactan (12 g.), after ether extraction, was dissolved in chloroform and fractionated by the portion-wise addition of light petroleum. The main fraction (8.1 g.) gave furfuraldehyde (3.1) on boiling with 12% hydrochloric acid. The last fraction (0.5 g.), on boiling with hydrochloric acid gave more furfuraldehyde (9.5). This fraction had a specific optical rotation $[\alpha]_D^{20}$ - 15 (methanol).

All fractions, except the last one, were combined, remethylated and hydrolysed. No pentose could be detected among the products of hydrolysis.

The last and arabinose-rich fraction was purified by extraction with boiling light petroleum. The residual white solid contained 19.4% dimethylaraban, calculated from furfuraldehyde estimation. After methanolysis and hydrolysis, 2:3:5-tri-O-methyl-L-arabinose was obtained in a yield of 8% of the arabinose-rich fraction, approximately one-third of the total pentosan content of the crude methylated polysaccharide. Besides there was 2:3:6-tri-O-methyl-D-galactose.

These arabofuranose residues were attached to the polysaccharide as terminal groups.

Polysaccharides containing both arabinose and galactose units have been found in a number of coniferous woods. These

arabogalactans are extracted from the wood with water. According to the definition given for hemicelluloses, these products should strictly speaking not belong to the hemicelluloses since they are originally water-soluble and do not require a preliminary treatment with alkali. But because of their structural similarities it is desirable to include them among the hemicelluloses.

These water-soluble polysaccharides are of interest in connection with the chemistry of wood formation. One of them is ε -galactan from larch which has been studied by a number of investigators. These results will, however, be described separately in the following chapter.

Schorger and Smith (7) pointed out in 1916 that most of the coniferae were characterized by the presence of galactans. Some fifteen years later Foreman and Englis (8) examined an arabogalactan from Southern yellow pine (Pinus palustris) and found 12.2% araban to be present.

Later on an investigation was carried out in California (9) on the arabogalactan from Jeffrey pine (Pinus Jeffrey) heartwood. The polysaccharide was extracted from pine sawdust with cold water and precipitated with ethanol to give a white powder, readily soluble in water with a specific optical rotation $[\alpha]_D^{23}$ + 17°. After hydrolysis of the polysaccharide in 0.1 N-sulphuric acid, the final specific rotation was + 88°,

which was consistent with that of a mixture of 5 moles of D-galactose and 4 moles of L-arabinose. The arabogalactan was methylated and, after methanolysis and subsequent hydrolysis, the products were separated on a column of powdered cellulose. The different fractions were 2:3:5-tri-O-methyl-L-arabinose (3 parts), di-O-methyl-L-arabinose (1 part), 2:3:4:6-tetra-O-methyl-D-galactose (0.3 parts), 2:3:4-tri-O-methyl-D-galactose (2 parts), 2:4-di-O-methyl-D-galactose (3 parts), mono-O-methyl-D-galactose (1 part) and trace of a partially methylated uronic acid. The relatively large proportions of tri-O-methyl-L-arabinose and di-O-methyl-D-galactose present in the arabogalactan indicated that the polysaccharide was highly branched.

The acid-catalyzed hydrolysis of polysaccharides to simple sugars is a widely known process. The reverse action produced by concentrated acids has been studied, and considerable attention has been focused on condensation polymerization and acid reversion products of monosaccharides.

For instance, the effect of hydrochloric acid on D-galactose has been examined (9a), and optimum polymerization occurred in a 1.0 M solution of D-galactose in 37% hydrochloric acid. Paper chromatographic examination revealed the presence of two disaccharides, a trisaccharide and a smaller proportion of higher saccharides in addition to D-galactose. The disaccharide

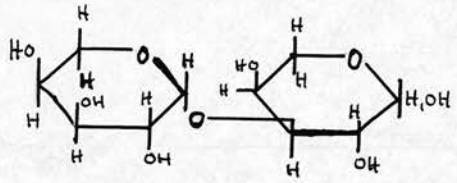
being present in greater amount than the other disaccharide was shown to be 6-O-α-D-galactopyranosyl-D-galactose.

Jones et al. (9b) have studied the effect of 6 N hydrochloric acid on D-xylose and L-arabinose. D-Xylose produced 4-O-β-D-xylopyranosyl-D-xylose among other disaccharides, one of which contained 1:5 linkage. A third disaccharide probably contained at least one xylofuranose residue.

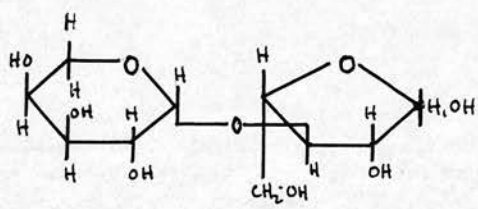
A similar experiment with L-arabinose produced several oligosaccharides, but the separation of these into pure components was found to be difficult. However, one disaccharide was identified as 4-O-β-L-arabopyranosyl-L-arabinose, from another fraction the osazone of 3-O-β-L-arabopyranosyl-L-arabinose was prepared, and a third disaccharide, probably 2-O-β-L-arabopyranosyl-L-arabinose, appeared to consist of two arabopyranose residues.

Some polysaccharides which have been found in different plant gums will be mentioned because they are of interest in connection with the investigation of ε-galactan.

After partial hydrolysis of ε-galactan, Jones (10) found among the hydrolysis products a disaccharide which he proved to be 3-O-β-L-arabopyranosyl-L-arabinose (V).



V.



VI.

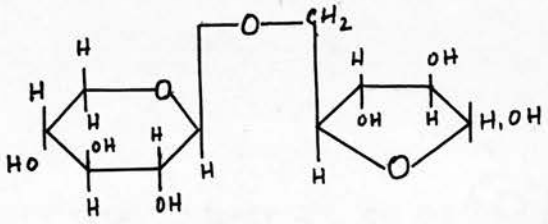
Recently Jones et al. found the same disaccharide in the hydrolysis products from golden apple (Spondias cytheria) gum (11) and from cherry and peach gum (12). These plant gums contain the following sugar residues:

	Cherry gum (13)	Peach gum (14)	Golden apple gum (11)
D-galactose	2 parts	5 parts	+
L-arabinose	6 parts	6 parts	+
D-xylose	ca. 1.5%	2 parts	+
D-mannose	1 part	-	-
L-rhamnose	-	ca. 2%	trace
Glycuronic acid	1 part	1 part	+

Methanolysis of methylated cherry gum (15) and fractional distillation of the products yielded the methyl glycosides of 2:3:5-tri-O-methyl-L-arabinose, 2:5-di-O-methyl-L-arabinose, 2:4:6-tri-O-methyl-D-galactose, 2:4-di-O-methyl-D-galactose, 2:3:4-tri-O-methyl-D-glucuronic acid, and 2:3-di-O-methyl-D-glucuronic acid. Di- and tri-O-methyl-L-arabinose were obtained in about equal amounts, and the ratio of di- and tri-O-methyl-D-galactose was also 1:1.

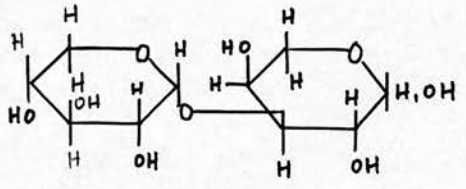
On partial hydrolysis of cherry and peach gum with N-sulphuric acid (12), xylose, arabinose and various oligo-

saccharides were produced. Chromatographic analysis of the hydrolysis products showed the presence of 3-O-β-L-arabopyranosyl-L-arabinose. Peach gum gave in addition a second disaccharide which probably was 5-O-β-L-xylopyranosyl-L-arabinose (VII).



VII.

Autohydrolysis of golden apple gum (11) yielded a mixture of reducing sugars consisting of arabinose, traces of galactose and rhamnose, a number of neutral pentose-containing oligosaccharides, acidic oligosaccharides, and the degraded gum. When the mixture was separated on a charcoal-Celite column, the main component of the oligosaccharide mixture was 3-O-β-L-arabopyranosyl-L-arabinose (V). The oligosaccharide next in quantity was 3-O-α-D-xylopyranosyl-L-arabinose (VIII).



VIII.

The arabinose unit in VIII could exist in the polysaccharide in either the pyranose or the furanose form. While the pyranose form is the most stable of the two, the furanose ring would immediately form the pyranose ring when its glycosidic linkage was broken during the hydrolysis.

Previous work had indicated that the majority of L-arabinose residues present in the gums and mucilages are in the acid-labile furanose form. But Jones *et al.*(10,11,12) have now shown that some of the L-arabinose units in polysaccharides occur in the pyranose form, and White (15a) has proved the presence of L-arabopyranose end-groups in Sapote gum.

It is becoming increasingly apparent that the plant-gums from the Prunus and Acacia genera have some common structural features (12). For example, the more acid resistant backbone of the gums, in which 1:3- and 1:6-linkages predominate, seems to be built up of D-galactose and uronic acid residues. The pentose side chains may resemble one another in certain important respects, notably in the presence of D-xylopyranose, L-arabopyranose, and L-arabofuranose residues.

The majority of the plant-gums contain L-arabofuranose, but some contain in addition also L-arabopyranose and D-xylopyranose. It has been considered probable that the pentose

sugars arise in nature from the corresponding hexoses by a process which involves oxidation to a uronic acid, followed by decarboxylation. D-Glucose and D-xylose are for instance closely associated in the cellulosic constituents of plants, and it is clear that D-xylose would be formed by the decarboxylation of D-glucuronic acid. But the presence of arabofuranose as terminal end-groups in the xylan molecule makes it more complicated, and the simple hypothesis of direct derivation from cellulose has been rendered uncertain.

A similar inter-relationship between galactose and arabinose would produce L-arabinose by the decarboxylation of D-galacturonic acid. D-Galactopyranose is almost invariably associated with L-arabinose in natural products, and D-arabinose has been found only in a few instances. One would have expected to find L-arabopyranose if the process was as simple as described, but apart from some exceptions one has found L-arabofuranose. It would be impossible, therefore, for the arabinose to arise intramolecularly from the associated galactose residue.

It seems as if the natural processes involve hydrolysis of one polysaccharide, transformation of the resulting hexose into the corresponding uronic acid or pentose, and lastly, synthesis of another polysaccharide from the uronic acid or pentose so formed (16).

At present little definite can be said regarding the inter-relationship of galactans, polygalacturonides and arabans, apart from the fact that D-galactose is almost invariably associated in natural products with L-arabinose.

It will be of interest to see whether or not L-arabopyranose residues occur in all arabinose-containing polysaccharides.

ε -Galactan.

The water-soluble polysaccharide ε -galactan can be isolated in 12-18% yield by water extraction of larch sawdust (17). The aqueous solution is evaporated to a smaller volume under reduced pressure, and the polysaccharide precipitated with ethanol. The product is filtered off, washed with ethanol and light petroleum, and, after drying, it gives a white amorphous powder, readily soluble in water, acid and dilute alkali.

Arabogalactans from several species of the larch family have been studied by a number of investigators. Campbell, Hirst and Jones (18) have examined ε -galactan from European larch (Larix decidua). The polysaccharide was a pale yellow

hygroscopic powder, easily soluble in water. The aqueous solution had low viscosity and gave no colour with aqueous iodine. It gave the following results on analysis. Ash, nil; $[\alpha]_D$ + 20 (approximately) in water; arabinose content (from furfuraldehyde determination), 12.7%; galactose on hydrolysis, 80%; uronic acid content, ca. 2%; iodine number (Bergman and Machemer method) corresponding to repeating unit of ca. 3000 equiv. wt. These figures are in general agreement with values recorded by other workers.

ε-Galactan, which is of interest in connection with the chemistry of wood formation, has also been studied for industrial purposes. The product can be extracted industrially at a reasonable cost, and it is proposed as a source of D-galactose, mucic acid, alcohol and industrial gum (19).

The polysaccharide was named by Schorger and Smith (7) who called it ε-galactan to differentiate it from earlier galactans, for instance the galactan (20) which was reported to be present in lucerne seeds. Steiger (21) investigated a product from the seeds of Lupinus albus. He called his product β-galactan and suggested the name α-galactan for the previously described galactan from lucerne seeds.

A γ-galactan was found in the lime water residues from sugar production (22), and δ-galactan was a water insoluble polysaccharide from agar-agar (22). Galactose was found to

be present in all these galactans, but little or nothing more was known about them.

ε-Galactan was first described by Trimble (24) in 1898 who showed that it contained galactose residues. Later Schorger and Smith (7) investigated a water soluble polysaccharide from Western larch (Larix occidentalis) with a specific optical rotation $[\alpha]_D^{20}$ + 12.11°. On distillation with 12% hydrochloric acid it yielded furfural corresponding to 10.5% pentosan, but they were unable to detect other sugar residues than D-galactose after hydrolysis of the polysaccharide.

Wise and Peterson (25) showed that the pentose present was arabinose, identified through its benzylphenylhydrazone and diphenylhydrazone. The amount of arabinose in the polysaccharide was 11.95%, calculated from distillation with 12% HCl and from arabinose diphenylhydrazone. The galactose was oxidised to mucic acid, the amount of which corresponded to 84.6% galactose. They adopted as a hypothesis that the polysaccharide actually was an arabogalactan with the condensed formula $[(C_5H_8O_4)(C_6H_{10}O_5)_6]_n$. Hydrolysis of such a polysaccharide would yield 11.9% arabinose and 88.1% galactose, which agree fairly well with the analytical data.

Wise and Unkauf (26) fractionated ε-galactan with ethanol from aqueous solution and got various fractions which were similar. ε-Galactan can also be obtained from Japanese (27),

Siberian (27a,28) and Eastern (29) larches and from larch wood from Honshu, Korea and Hokkaido (30).

When methylation studies started on ε-galactan, doubt arose to whether the polysaccharide was homogeneous or not. All the papers published on the subject up to about 1940 seemed to agree that ε-galactan is a true arabogalactan and not a mixture of several polysaccharides. During the last 15 years a number of investigators have examined ε-galactan and different results have been obtained. But no clear decision has been reached yet as to whether it is a single polysaccharide of the arabogalactan class or whether it is a mixture of polysaccharides, one of which is a true galactan.

Peterson et al. (31) isolated water soluble polysaccharides from Eastern, Western, and European larches, all of which gave similar analysis for ash, reducing value, optical activity, anhydro-arabinose and anhydro-galactose, which however did not establish their chemical identity.

By systematic fractionation of the acetyl, the propionyl and the benzoyl esters of Western larch from various solvent mixtures, they obtained fractions of similar acyl content but of variable optical activity, reducing value, specific viscosity and araban content. The araban content of the fractions varied between 5.5% - 9.6% for the propionates, 3.7% - 6.1% for the benzoates, and 5.5% - 7.7% for the acetates.

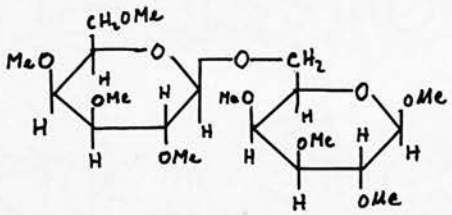
It was concluded that these derivatives were non-homogeneous and that the original polysaccharide probably also was a mixture.

The methyl ethers of an arabogalactan from Japanese larch (Larix leptolepis, Gord.) have been studied by Tachi and Yamamori (27). The water soluble polysaccharide had the same characteristics and chemical composition as the ε-galactan reported by other workers. The fully methylated product with a specific optical rotation $[\alpha]_D^{15}$ -32.94° in acetone was subjected to vigorous fractionation for a test of homogeneity by stepwise addition of light petroleum to a solution of the methylated polysaccharide in acetone. Four fractions were obtained, all of which had similar methoxyl content, specific rotation and amount of araban. The araban content varied between 5.1% - 5.7%. These two research workers found it possible to assume that ε-galactan is homogeneous with the formula suggested by Wise and Peterson (25).

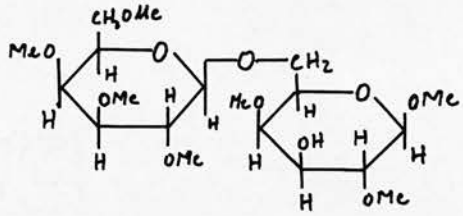
The same conclusion had been reached by White through his work on the arabogalactan from Western larch (32). The fully methylated polysaccharide after methanolysis and fractional distillation gave the methyl glycosides of

2:3:5-tri-O-methyl-L-arabofuranose	1 mole
2:3:4:6-tetra-O-methyl-D-galactose	2 moles
2:3:4-tri-O-methyl-D-galactose	1 mole
2:4-di-O-methyl-D-galactose	3 moles.

On partial methanolysis of the methylated polysaccharide the reaction products were separable into two fractions with hot petroleum ether. In the soluble fraction he found besides the methyl glycosides of 2:3:5-tri-O-methyl-L-arabofuranose, 2:3:4:6-tetra-O-methyl-D-galactose, and 2:3:4-tri-O-methyl-D-galactose, two high-boiling disaccharides, namely octamethyl-6-O-β-D-galactosyl-D-galactose (IX) and heptamethyl-6-O-β-D-galactosyl-D-galactose (X).



IX.



X.

In heptamethyl-6-O-β-D-galactosyl-D-galactose the galactose unit linked through carbon C6 possessed an hydroxyl group at carbon C3, which showed that this was a branch point. The low positive rotation of the disaccharides indicated that they were β-linked.

The fraction which was insoluble in petroleum ether gave on complete methanolysis mainly 2:4-di-O-methyl-D-galactoside and only some 2:3:4:6-tetra-O-methyl-D-galactoside. No

tri-O-methyl-D-galactoside could be detected among the alcoholysis products. When this fraction was remethylated and then hydrolysed, one found 2:3:4-tri-O-methyl-D-galactose and 2:4:6-tri-O-methyl-D-galactose in addition to 2:3:4:6-tetra-O-methyl-D-galactose and 2:4-di-O-methyl-D-galactose.

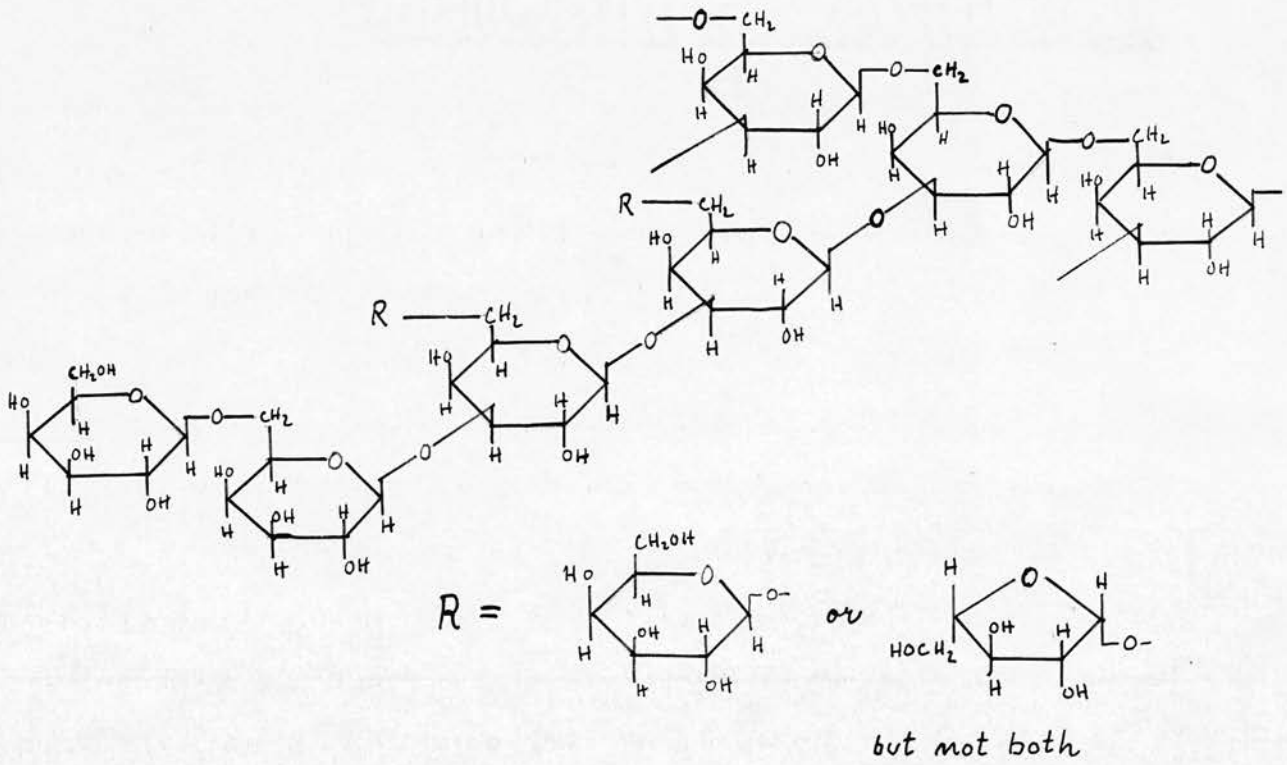
White hydrolysed ε-galactan with 0.02 N-sulphuric acid at 90° and followed the decrease of arabinose in the residual polysaccharide which was precipitated from the solution with ethanol. Thus after 23 hours the amount of residual polysaccharide was 88.4% containing 6.16% arabinose. After methylation of this degraded polysaccharide and subsequent methanolysis he got equimolecular proportions of the methyl glycosides of 2:3:4:6-tetra-O-methyl-D-galactose, tri-O-methyl-D-galactose, and 2:4-di-O-methyl-D-galactose. The tri-O-methyl-D-galactoside fraction was a mixture of 2:3:4-tri-O-methyl-D-galactoside and 2:4:6-tri-O-methyl-D-galactoside. No attempt was made to estimate the methylated arabinose which was present.

White's opinion was that the L-arabofuranose unit was linked to the original polysaccharide to carbon C6 in a D-galactose unit whose carbon C1 and C3 were linked to other units. The presence of 2:4:6-di-O-methyl-D-galactose in the degraded polysaccharide proved this.

The ease of hydrolysis of L-arabinose indicated that it was present in its furanose form.

The two disaccharides obtained on partial methanolysis of the methylated ε-galactan gave evidence for the presence of the linkage Ga1-6Ga to be present between two D-galactopyranose units. Since 1:6-bonds are usually more resistant to hydrolysis than others, they tend to accumulate on hydrolysis. In fact, during the early stages of methanolysis there accumulated a resistant residue composed of 1:6 linked 2:4-di-O-methyl-D-galactose units.

On the basis of these data, White proposed that the molecule is highly branched with L-arabofuranose units attached as end units on some of the branches. He suggested the following structure (XI)



XI.

Campbell, Hirst and Jones (18,33) had strong evidence, however, that the material from European larch (Larix decidua) was a mixture of polysaccharides, one of which was a true galactan, and the other either an arabogalactan or an araban with a galactan differing in structure from the first one. They found that ε-galactan was readily methylated by the thallous ethoxide-methyl iodide method. The methylated product (20.5 g.) had a methoxyl content 42.9% and was easily fractionated from acetone solution by the portionwise addition of light petroleum. The first fraction (13 g.) had a specific rotation $[\alpha]_D$-18° in methanol, 42.5% methoxyl and the araban content on boiling with 12% hydrochloric acid was less than 2%. The second fraction (4 g.) had the same methoxyl content, specific rotation $[\alpha]_D$-19° and gave 4.7% furfuraldehyde corresponding to 12% dimethylaraban. Fraction three (3 g.) had [α]-16° and contained 44.3% methoxyl. Some sticky solid was left which was not further examined.

The first fraction after methanolysis and subsequent hydrolysis gave equimolecular proportions of 2:3:4:6-tetra-O-methyl-D-galactose, 2:3:4-tri-O-methyl-D-galactose and 2:4-di-O-methyl-D-galactose.

A degraded product was obtained on hydrolysis with 0.01 N-sulphuric acid at 90° for 40 hours. After neutralization with barium carbonate and concentration of the filtrate, the

remaining syrup was fractionated by the addition of alcohol. The main fraction, which had a specific rotation $[\alpha]_D$-18° in methanol and contained 2.9% araban on boiling with 12% hydrochloric acid, was hydrolysed completely with N-sulphuric acid. Trituration with alcohol gave crystalline D-galactose in 85% yield; no other sugar could be detected.

This degraded polysaccharide was methylated and after hydrolysis of the methylated product, 2:3:4:6-tetra-O-methyl-D-galactose, 2:3:4-tri-O-methyl-D-galactose, 2:4:6-tri-O-methyl-D-galactose, and 2:4-di-O-methyl-D-galactose were obtained.

The investigators say that these observations provide strong evidence in favour of the heterogeneity of the original ε-galactan. The results from the fractionation of the methylated undegraded product showed that there must be one true galactan present. The three D-galactopyranose units which make up the repeating unit of the galactan molecule are, respectively, a terminal D-galactose residue attached only through C1 (Ga1...), a residue attached to others through C1, C3, and C6 ($\cdots\overset{3}{\underset{6}{\cdots}}$ Ga1...) and a residue attached to the others through C1 and C6 (...6Ga1...). The possible structure of the β-linked galactopyranose units would thus be (XII)

$$\cdots \left[\begin{matrix} \overset{3}{\underset{1}{,6}}\text{Ga1, 6Ga1, } \cdots \\ \text{Ga} \end{matrix} \right]_n \cdots$$

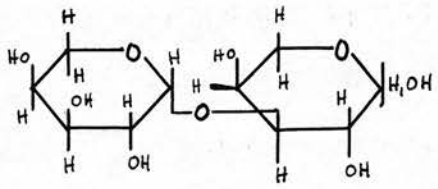
Ga = β-D-galactopyranose residue

XII.

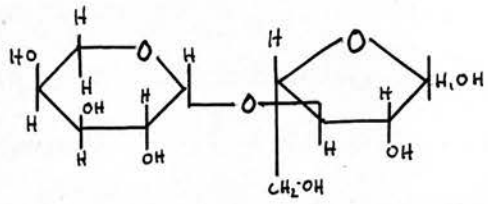
This type of structure is similar to that present in plant gums and reveals a striking resemblance between this galactan and the araban of pectic materials. The ring structure of the constituent sugars is so different, however, that the arabofuranose residues in the araban could not arise from such a galactan through oxidation and decarboxylation only.

Therefore, it was very interesting indeed when Jones (10) found the occurrence of L-arabopyranose among the products obtained on partial hydrolysis of larch ε-galactan. The polysaccharide was hydrolysed with 0.01 N-hydrochloric acid at 100° for 1½ hours, neutralized with ion exchange resin, and poured into alcohol. The precipitated, degraded ε-galactan was collected and the filtrate evaporated to a syrupy mixture of sugars which was fractionated on a column of cellulose with n-butanol half saturated with water. L-Arabinose appeared first in the effluent followed by D-galactose, a trace of an unidentified pentose-containing oligosaccharide, 3-O-β-L-arabopyranosyl-L-arabinose (XIII), and then a hexose-containing disaccharide. The disaccharide 3-O-β-L-arabopyranosyl-L-arabinose gave rise to 2:3:4-tri-O-methyl-L-arabinose and 2:4-di-O-methyl-L-arabinose after methylation and subsequent hydrolysis. It probably existed as (XIV) in ε-galactan and

may have had L-arabofuranose residues attached to the L-arabopyranose portion of the disaccharide.



XIII.



XIV.

In order to prove that the disaccharide did not arise by resynthesis, a solution of L-arabinose in 0.01 N-hydrochloric acid was heated at 100° for 1½ hours. No oligosaccharides were produced.

Brown, Dunstan et al. (34) carried out periodate oxidations on ε-galactan and found three end groups per repeating unit of mol. wt. 1104.

Heidelberger (35) has examined ε-galactan from European larch by means of quantitative immunochemical methods which have been of great service in the clarification of structural chemical relationship. He measured the antibody nitrogen precipitated in Type XIV antipneumococcal horse sera by a number of polysaccharides containing galactose. A galactan

isolated from beef lungs precipitated more than one quarter of the antibody in a Type XIV antipneumococcus serum. This demonstrated a close relationship between the galactose linkages in the galactan and the galactose linkages in the immunologically specific polysaccharide of Type XIV pneumococcus.

ε-Galactan (88% galactose, 12% arabinose) and degraded ε-galactan (92% galactose, 8% arabinose) were both made to react with Type XIV antipneumococcus horse serum. The amount of antibody nitrogen precipitated was about the same for both products (slightly less for the degraded material) which showed that there was little difference before and after removal of one third of arabinose in the original polysaccharide.

Heidelberger determined the amount of galactose and arabinose in the precipitates (36). The ratio galactose: arabinose in undegraded and degraded ε-galactan was maintained in their cross reaction precipitates. This showed that no fractionation of either of the two polysaccharides had taken place. But Heidelberger says that only a positive result is meaningful; a negative result does not show that a substance is homogeneous.

Some investigators have used physical methods in their

work on larch galactan. Husemann (37) found from osmotic pressure and viscosity measurements an average degree of polymerization of 222.

Husemann fractionated ε-galactan from an aqueous calcium chloride solution by the portionwise addition of methanol. She obtained five fractions where the degree of polymerization varied between 267 and 182 for the first and last fraction, the molecular weight lying between 42000 and 28600. No fractionation in an araban and a galactan had taken place, which would have been expected if they did not possess the same solubility. She found the Staudinger constant for ε-galactan to be about the same as that for starch. She says that if the polysaccharide is a true arabogalactan it is highly branched and consists of chains of different length.

Owens proposed a molecular weight of at least 2200 on the basis of Staudinger 's equation, which agrees well with that of Nikitin and Soloviev (27a) who obtained 2208 by chemical methods. Arabogalactan was found to have spherical shape when dissolved in water.

Studies by Mosimann and Svedberg (39) using the sedimentation velocity centrifuge technique indicated that ε-galactan consisted of two components with molecular weights of 100,000 and 16,000.

Gerd Lystad Borgin (40) examined water soluble extracts from the sapwood and the heartwood of Western larch. Sedimentation velocity runs showed heartwood extract to be made up of one component, whereas the sapwood was made up of two components. The two components were estimated to be present in the proportions of 3:2, the component of higher molecular weight being present in the larger amount.

The following investigations were carried out on ε-galactan from European larch (Larix decidua). The material is the same as that investigated by Heidelberger (35,36) and by Jones et al.[10,18] The original ε-galactan as well as a degraded product were examined and the hydrolysis products separated by means of partition chromatography. It was hoped that these new and delicate methods which now are in general use in carbohydrate chemistry would make it possible to bring some further insight into the fine structure of ε-galactan.

E X P E R I M E N T A L

EXPERIMENTAL

NOTES

1. Paper chromatograms were run on the paper Whatman No.1, unless otherwise stated.

2. A saturated aqueous solution of aniline oxalate was used for spraying the paper chromatograms, unless otherwise stated.

3. The paper chromatograms were run in one of the following solvents:
   a) n-butanol-ethanol-water (4:1:5), top layer,
   b) n-butanol-ethanol-water (40:11:19),
   c) ethyl acetate-pyridine-water (10:4:3),
   d) benzene-ethanol-water (169:47:15), top layer,
   e) n-butanol-formic acid-water (500:115:385), top layer.

4. Melting point determinations were carried out on a Kofler block, and no corrections were made.

5. Methoxyl estimations were carried out by the Zeisel volumetric method.

General Properties of ε-Galactan and Degraded ε-Galactan

The polysaccharide ε-galactan was prepared at Forest Products Research Laboratory, Princes Risborough, Bucks., where it had been extracted from the sawdust of European larch (Larix decidua) with water. The polysaccharide was purified by passage through Amberlite resins IR120 and IR400 and was recovered from solution by precipitation with alcohol.

The degraded ε-galactan was a mixed batch. It was composed of two preparations of ε-galactan, one from Princes Risborough, and one prepared by Dr. J.K.N. Jones, Bristol, from larch sawdust provided by Princes Risborough. The polysaccharides had each been given two treatments with boiling 0.01N hydrochloric acid (1 $\frac{1}{2}$ hours) followed by passing the solution through a column of Amberlite IR-4B(OH) to remove hydrochloric acid. They were recovered by precipitation in alcohol.

Both polysaccharides are pale yellow hygroscopic powders, readily soluble in water, acid, and dilute alkali.

The optical rotation is $[\alpha]_D^{17}+19°$. ($\underline{c}$ = 0.2 in water) for undegraded, and $[\alpha]_D^{17}+19°$ ($\underline{c}$ = 0.5 in water) for degraded product (a stronger solution made the polarimeter reading impossible).

The two polysaccharides are easily hydrolysed in dilute

mineral acid. Complete hydrolysis is obtained by heating the products with N-sulphuric acid at 100°C (4 hours).

Preliminary experiments with paper chromatography showed that both polysaccharides gave two spots with R_G values corresponding to those of arabinose and galactose.

Estimation of Galactose and Arabinose by Quantitative Paper Chromatography.

The polysaccharides (20-40mg.) were hydrolysed with N-sulphuric acid (1ml.) in a sealed tube for four hours in the boiling water-bath. L-Rhamnose (10-20mg.) being used as reference sugar was added to the solution after the hydrolysis. The solution was then neutralised with barium carbonate. The precipitated barium sulphate was filtered off, washed with water, and the filtrates evaporated to a syrup in a vacuum dessiccator over phosphoric oxide.

The separation of the sugars was carried out by the method of Flood, Hirst and Jones (41) on paper chromatograms run in solvent (a) for two days. The amount of each sugar was estimated by oxidation with sodium meta-periodate (42). Sodium meta-periodate solution (1ml.;0.3M) was added to the sugar solution (5ml.) in a boiling tube with ground in stopper. The stoppered tube was heated in the boiling

water-bath (20 minutes), the side of the tube being cooled by a water coil-condenser.

After the tube had been heated the requisite length of time it was cooled under the tap, ethylene glycol (ca. 0.2ml.) added to destroy excess of periodate, and the liberated formic acid titrated with sodium hydroxide (0.01N), using methyl-red as indicator.

Paper blank was run each time.

The amount of sugar (mg.) in each tube was found according to the formulae:

$$\text{hexose} = \frac{N_{NaOH} \times ml_{NaOH} \times M_{hexose}}{5}$$

$$\text{pentose} = \frac{N_{NaOH} \times ml_{NaOH} \times M_{pentose}}{4}$$

N = normality of sodium hydroxide

ml_{NaOH} = titre sodium hydroxide

M_{hexose} = molecular weight of hexose

$M_{pentose}$ = molecular weight of pentose.

The amount of galactose and arabinose found was calculated as per cent anhydrosugar present in the polysaccharide.

Undegraded ε-galactan

Wt. (mg.) polysacch.	Wt. (mg.) rhamnose	Titre(ml.), corrected for blank galactose	arabinose	rhamnose
23.23	9.02	4.05	0.59	1.47
23.23	9.02	3.62	0.50	1.30
29.81	14.63	4.96	0.65	2.27

	Galactan %	Araban %
	84.5	12.5
	85.5	12.0
	84.8	11.3
Mean =	84.9	11.9

Degraded ε-galactan

Wt. (mg.) polysacch.	Wt. (mg.) rhamnose	Titre(ml.), corrected for blank galactose	arabinose	rhamnose
25.38	20.82	6.42	0.61	4.71
25.38	20.82	3.16	0.27	2.33
20.67	10.28	5.35	0.47	2.37

	Galactan %	Araban %
	88.4	8.5
	87.9	7.7
	88.7	7.9
Mean =	88.3	8.0

In the undegraded ε-galactan the two monosaccharides were found in six to one molecular ratio. If we adopt as a hypothesis that the polysaccharide is a homogeneous arabo-galactan the empirical formula would then be $[(C_5H_8O_4)(C_6H_{10}O_5)_6]_n$.

In the degraded ε-galactan the two monosaccharides were found in nine to one molecular ratio (or in six to two-thirds) and accordingly the degraded polysaccharide can be given the empirical formula $[(C_5H_8O_4)_{\frac{2}{3}}(C_6H_{10}O_5)_6]_n$.

Partial Hydrolysis.

Hydrolysis with 0.5N sulphuric acid.

Undegraded polysaccharide (5g.) was heated with 0.5N sulphuric acid under reflux in the boiling water-bath. Every half hour a sample (3ml.) was withdrawn, neutralised with barium carbonate, filtered and concentrated. Each sample was examined on a paper chromatogram using the solvent (c). The paper was sprayed with p-anisidine-hydrochloride.

The results are tabulated below. The numbers in brackets are the distance (cm.) each spot travelled from the starting line.

Time (hrs.)	Spots
0.5	Brown(7), pink(15), galactose(18), arabinose(26).
1.0	Five brown spots (1.5, 3, 2, 7, 9), pink(15), galactose(18), arabinose(26).
1.5	Five brown spots, galactose, arabinose.
28	As for 1.5 hours.

The pink spot that appeared just above galactose was probably the disaccharide 3-O-β-L-arabopyranosyl-L-arabinose(10). It disappeared on prolonged heating which showed that it was hydrolysed to arabinose.

Hydrolysis with 0.01N sulphuric acid.

Degraded polysaccharide (0.5g.) was heated under reflux with 0.01N sulphuric acid (5ml.) in the boiling water-bath. At intervals (4 hours) the solution was neutralised by shaking with resin Amberlite IR 4B(OH), filtered, and the partially hydrolysed polysaccharide precipitated with alcohol. The polysaccharide was filtered off, washed thoroughly with alcohol and again hydrolysed with dilute acid and given the same treatment as before.

Each filtrate was concentrated to a syrup under reduced pressure and examined on the paper chromatogram run in solvent (c). All the filtrates gave the same spots on the chromatogram, namely arabinose, galactose, a pink spot above galactose (probably 3-O-β-L-arabopyranosyl-L-arabinose), and a slower travelling brown spot.

After five treatments (20 hours hydrolysis) nothing remained of the polysaccharide after the hydrolysis. The solution, after neutralisation, was however examined on a paper

chromatogram and gave the same spots as mentioned above.

The amount of araban and galactan present in the partially hydrolysed polysaccharides was estimated (41,42) after a sample had been completely hydrolysed in N-sulphuric acid.

Time (hrs.)	Araban %	Galactan %
4	6.7	91.4
8	4.6	89.1
12	3.0	89.6
16	1.2	94.0

Periodate Oxidation Experiments.

Determination of formic acid released.

Undegraded and degraded polysaccharides were oxidised by periodate ion by the method of Halsall, Hirst, and Jones (58), and the amount of formic acid liberated estimated by titration with 0.01N sodium hydroxide.

The polysaccharide (100mg.) was dissolved in water (30ml.) in a stoppered bottle. Potassium chloride solution (10ml.;16%) and sodium meta-periodate (20ml.;0.3M) were added, and the bottle was shaken continuously in the dark. Blank was run at the same time only omitting the polysaccharide. At regular intervals the shaking was stopped and a sample (5ml.) withdrawn,

excess of periodate destroyed by the addition of ethylene glycol (1ml.; neutral to methyl red), and the amount of formic acid liberated titrated with sodium hydroxide (0.01N) from a micro burette, using methyl red.

100.08mg. undegraded ε-galactan. Normality NaOH;0.01054

Time (hrs.)	ml.NaOH/ 5ml. solution	Moles HCOOH/ $(C_5H_8O_4)(C_6H_{10}O_5)_6$
1	0.84	1.2
25	2.02	2.8
95	2.43	3.4
171	2.54	3.5
262	2.71	3.8
359	2.78	3.9
432	2.82	3.9
555	2.82	3.9

109.67mg. degraded ε-galactan.

Time(hrs.)	ml.NaOH/ 5ml. solution	Moles HCOOH/ $(C_5H_8O_4)_{\frac{2}{3}}(C_6H_{10}O_5)_6$
1	1.17	1.4
25	2.51	3.1
95	2.86	3.6
171	3.02	3.8
262	3.12	3.9
359	3.28	4.1
432	3.32	4.2
555	3.32	4.2

An experiment in which the bottles were not shaken, was carried out. The reaction seemed to go slower in this case. The results are tabulated below.

100.25mg. undegraded ε-galactan. Normality NaOH;0.01054

Time (hrs.)	ml.NaOH/ 5ml. solution	Moles HCOOH/ $(C_5H_8O_4)(C_6H_{10}O_5)_6$
1	0.82	1.4
22	1.33	1.8
67	2.02	2.8
121	2.33	3.2
188	2.48	3.4
240	2.54	3.5
287	2.58	3.6
334	2.58	3.6
427	2.72	3.8
528	2.81	3.9

104.59mg. degraded ε-galactan.

Time (hrs.)	ml.NaOH/ 5ml. solution	Moles HCOOH/ $(C_5H_8O_4)_{\frac{2}{3}}(C_6H_{10}O_5)_6$
1	0.81	1.6
22	1.52	2.0
67	2.24	2.9
121	2.58	3.4
188	2.72	3.6
240	2.77	3.7
287	2.83	3.7
334	2.89	3.8
427	3.00	4.0
528	3.01	4.0

Uptake of periodate.

The uptake of periodate was estimated by the following method (59,60). The polysaccharide (100-200mg.) was dissolved in water (75ml.) and sodium meta-periodate solution (25ml.;0.3M) added. Blank was run at the same time only omitting the polysaccharide. The stoppered reaction-flasks were kept in diffused light. A sample (5ml.) was withdrawn periodically, diluted with water (50ml.), followed by a saturated aqueous borax solution (5ml.), and solid boric acid (2g.). The solution was mixed by swirling, potassium iodide solution (5ml.;40%) added, and the flask stoppered. After 3-4 minutes the liberated iodine was titrated with standard arsenite solution (0.05N), starch being used at the end-point.

108.05mg. undegraded ε-galactan. Normality As_2O_3; 0.05001

Time (hrs.)	Titre (ml.)	Moles periodate/ $(C_5H_8O_4)(C_6H_{10}O_5)_6$
24	1.50	7.7
70	1.55	7.9
117	1.56	8.0
140	1.59	8.1
332	1.59	8.1

100.55mg. degraded ε-galactan.

Time (hrs.)	Titre (ml.)	Moles periodate/ $(C_5H_8O_4)\frac{2}{3}(C_6H_{10}O_5)_6$
24	1.47	7.7
70	1.50	7.9
117	1.59	8.3
140	1.62	8.5
332	1.66	8.7

Ethylene glycol was added to the solutions containing the fully oxidised residues, which were then dialysed in plastic tubings against running water (7 days). After concentration *in vacuo* the residues were hydrolysed with N-sulphuric acid and examined by paper chromatography using the solvent (c). Galactose, arabinose, and a pink spot below arabinose (R_G value as xylose) were found.

Summary of results.

	Moles HCOOH/ unit	Moles periodate/ unit
$(C_5H_8O_4)(C_6H_{10}O_5)_6$	3.9	8.1
$(C_5H_8O_4)\frac{2}{3}(C_6H_{10}O_5)_6$	4.1	8.7

Since hexose residues linked through carbon C1 only, or through C1 and C6, produce formic acid on oxidation, the results

indicated that the polysaccharides either contained 1-6' linked hexose residues, or possessed a high degree of branching. A pentose residue linked through carbon C1 would be oxidised by the periodate ion, the pentofuranoside would require one mole periodate, while the pentopyranoside would require two moles periodate and produce one mole of formic acid.

The presence of arabinose in the hydrolysis products of the oxidised polysaccharides indicated that this sugar cannot be present entirely as end groups.

The similar results for the undegraded and degraded polysaccharide showed that there is little difference before and after removal of one-third of the arabinose.

However, the results are of value only when taken in conjunction with the results of methylation studies.

Degradation with phenylhydrazine acetate (Barry's method).

The two polysaccharides (4g.) were dissolved in water (50ml.) and sodium meta-periodate solution (150ml.;0.3M). At intervals a sample (1ml.) was withdrawn and the liberated formic acid and the periodate consumption estimated as previously described. Blank was run at the same time only omitting the polysaccharide.

$(C_5H_8O_4)(C_6H_{10}O_5)_6$ = AG_6

Time (hrs.)	HCOOH/ AG_6	HCOOH/ g.polysacch.	Moles IO_4/ AG_6	Moles IO_4/ g.polysacch.
4	3.2	1.5	7.5	3.4
25	3.5	1.6	8.1	3.7
51	3.7	1.7	8.3	3.8
120	4.0	1.8	8.7	4.0
168	4.1	1.8	9.0	4.1

$(C_5H_8O_4)_{\frac{2}{3}}(C_6H_{10}O_5)_6$ = $A_{\frac{2}{3}}G_6$

Time (hrs.)	HCOOH/ $A_{\frac{2}{3}}G_6$	HCOOH/ g.polysacch.	Moles IO_4/ $A_{\frac{2}{3}}G_6$	Moles IO_4/ g.polysacch.
4	3.2	1.5	7.8	3.7
25	3.5	1.7	8.1	3.9
51	3.6	1.7	8.3	4.0
120	3.9	1.8	8.9	4.2
168	4.1	1.9	9.0	4.3

Ethylene glycol was added to the two solutions which were dialysed in plastic tubings against running water (14 days).

After concentration in vacuo alcohol was added in order to precipitate the oxidised polysaccharide. Some material was precipitated, but it soon went into solution again. Further addition of alcohol was of no use. The solutions were

concentrated under reduced pressure to smaller volumes (50ml.).

The oxidised material was degraded with phenylhydrazine acetate according to the method by O'Colla (63). Phenylhydrazine acetate was prepared (64) by adding phenylhydrazine (15g.) dropwise to glacial acetic acid (9g.) under stirring at -7°C. The crystals were filtered off and washed with cold, dry ether.

The solution containing the oxidised polysaccharide was heated with phenylhydrazine acetate (10g.) under reflux on the boiling water-bath for one hour. At the end of this time a brown gum had precipitated in the flask. Addition of alcohol precipitated more of the gelatinous product which solidified on washing with alcohol and hot water. The amorphous brown powder (polysaccharide) was filtered off at the pump and washed with alcohol and ether. Yield = 2.2g. for both.

Hydrolysis of the brown powder in N-sulphuric acid followed by paper chromatography showed the presence of galactose, arabinose, and a faster travelling pink spot.

Second periodate oxidation.

The amorphous brown polysaccharides (1.0g.) were dispersed in water (25 ml.) and sodium meta-periodate solution (50ml.;0.3M). The stoppered reaction flasks were shaken in the dark. A suspension of the polysaccharides in water was neutral to methyl red.

ε -Galactan.

Time (hrs.)	HCOOH/ g.polysacch.	Moles IO_4/ g.polysacch.
3	0.3	2.5
24	0.7	3.0
74	1.2	4.5
143	1.7	5.7
191	2.0	6.5
241	2.2	6.5
313	2.5	7.3

Degraded ε-galactan.

Time (hrs.)	HCOOH/ g.polysacch.	Moles IO_4/ g.polysacch.
3	0.3	2.5
24	0.8	3.4
74	1.3	4.6
143	1.9	6.3
191	2.2	6.9
241	2.4	7.5
313	2.7	8.4

The insoluble polysaccharide was filtered off and washed with water and alcohol. Weight = 0.85g.

Ethylene glycol was added to the filtrates which were dialysed against running water (6 days). Concentration followed by hydrolysis (N-sulphuric acid) and paper chromatography showed the presence of galactose, arabinose, together with two pink, three brown, and one grey faster travelling spots.

The insoluble periodate-oxidised polysaccharide (0.85g.) was again heated with phenylhydrazine acetate (1g.) in water (20ml.) on the boiling water-bath for one hour. The solution became brown but the polysaccharide did not dissolve. Insoluble material was filtered off and washed with water and alcohol. Weight = 0.75g.

Third periodate oxidation.

The brown insoluble products which were left after the second treatment with phenylhydrazine acetate were again oxidised by the periodate ion. The material (0.63g.) was dispersed in water (25ml.) and sodium meta-periodate solution (50ml.;0.3M) and shaken in the dark. A sample (1ml.) was withdrawn periodically and formic acid released and periodate consumption estimated in the usual way.

ε -Galactan.

Time (hrs.)	HCOOH/ g.polysacch.	Moles IO_4/ g.polysacch.
4	0.6	1.8
24	1.0	2.6
51	1.3	3.1
120	1.9	5.0
192	2.6	6.9
288	3.3	7.3
456	4.6	9.8
3 Months	13.2	25.9

Degraded ε -galactan.

Time (hrs.)	HCOOH/ g.polysacch.	Moles IO_4/ g.polysacch.
4	0.5	3.4
24	1.2	3.8
51	1.6	4.6
120	2.2	6.3
192	2.8	7.5
288	3.7	9.1
456	4.9	11.5
3 Months	13.2	26.0

The insoluble material was filtered off the brown solution and washed with water and alcohol. Weight = 0.2g.

The polysaccharides were hydrolysed with N-sulphuric acid. Examination on the paper chromatogram showed the presence of galactose and arabinose.

The filtrates were dialysed, concentrated, and hydrolysed in the usual way. On a paper chromatogram there were spots of galactose, arabinose, and a faster moving pink spot (same R_G value as xylose).

Estimation of arabinose and galactose.

The brown amorphous polysaccharides which were obtained after the periodate-oxidised material had been treated with phenylhydrazine acetate were analysed for arabinose and galactose. This was carried out by hydrolysis with N-sulphuric acid and quantitative paper chromatography as previously described. The brown solutions which were obtained after neutralisation with barium carbonate were decolourised by charcoal.

ε-Galactan.

	Galactan %	Araban %
First IO_4-oxidation	27.0	4.5
Second IO_4-oxidation	31.7	2.9

Degraded ε-galactan.

	Galactan %	Araban %
First IO_4-oxidation	25.1	2.8
Second IO_4-oxidation	29.2	1.7

Condensation with isonicotinoylhydrazide (65).

ε-Galactan (0.3g.) was dissolved in water (25ml.) and sodium

meta-periodate solution (25ml.;0.3M) and oxidised for 168 hours. Ethylene glycol was added at the end of this time, and the solution dialysed (10 days), and concentrated under reduced pressure (25ml.). An aqueous solution of "isoniazide" (0.90g. in 50ml. water) was added. The solution became cloudy, and it was necessary to add a little sodium chloride to effect the condensation product to separate.

The pale yellow precipitate was filtered off after two days and washed with water, alcohol, and finally ether.

The filtrate was dialysed (7 days), and then concentrated in vacuo.

Hydrolysis with N-sulphuric acid of the precipitate and the filtrate, followed by paper chromatography, showed the presence of galactose, arabinose, and a pink spot (R_G value as xylose) in both samples.

Condensation with phenylhydrazine (66).

Undegraded and degraded polysaccharides (1.7g.) were oxidised with sodium meta-periodate for 144 hours as before. The solutions were treated with lead acetate to remove iodate and periodate, and then with dilute sulphuric acid to precipitate excess of lead. The solution of oxygalactan obtained (160ml.) was treated at room temperature with phenylhydrazine (3.5ml.) in 10% acetic acid (10ml.). The yellow solid obtained was washed well with water and dried to a yellow powder (1.5g.).

Degradation of oxygalactan-phenylhydrazine derivative.

Undegraded ε-galactan.

The yellow solid (1.5g.) was suspended in ethanol (40ml.), phenylhydrazine (5ml.) in glacial acetic acid (8ml.), and water (15ml.). The product dissolved on heating and was heated under

reflux on the boiling water-bath for 4 hours.

Removal of the ethanol under reduced pressure precipitated crystalline material. This was filtered off, washed with water, dilute acetic acid, water, and finally dried to yield orange crystals (A, 1.7g.).

The filtrate was extracted with ether, benzene, and again ether. Concentration of the aqueous filtrate *in vacuo* yielded red gum (B, 0.6g.). Hydrolysis in N-sulphuric acid followed by paper chromatography showed the presence of arabinose and galactose.

The benzene-ether extracts were concentrated to yield a deep red solution (C).

The crystalline precipitate (A, 1.7g.) was dissolved in benzene and adsorbed on a column of alumina (80g.). The column was eluted with benzene, benzene:ether (1:1), ether, and finally ethanol:water (9:1). The solvent was distilled off the fractions under reduced pressure. The residue was weighed without further drying, which accounts for the too high yield.

Solvent	Fractions	Volume (ml.)	Weight (g.)
Benzene	1	100	1.00
"	2-3	150	0.20
Benzene:ether	4	50	0.01
"	5-7	300	0.12
Ether	8-9	175	0.36
Ethanol:water	10	30	0.41
"	11	20	0.04
"	12	40	0.01

Fractions 1-3 were recrystallised from benzene to give pale yellow blades, m.p.168°C., not depressed by admixture with an authentic specimen of glyoxalbisphenylhydrazone (m.p.168°C).

Fractions 5-7 were recrystallised from benzene, and gave colourless blades, m.p.128°C., not depressed by admixture with an authentic specimen of N-acetylphenylhydrazine (129°C).

The mother liquor crystallised further to give deep yellow crystals which after recrystallisation from benzene had m.p.130°C., glycerosazone, depressed by admixture with N-acetylphenylhydrazine. The melting point was not depressed by admixture with an authentic specimen of glycerosazone (m.p.128°C.).

Fractions 8-9. These fractions came almost colourless out of the column. Removal of the solvent and recrystallisation from benzene yielded colourless blades, m.p.130°C., not depressed by admixture with an authentic specimen of N-acetylphenylhydrazine.

Fraction 10. Recrystallisation from benzene gave colourless blades of N-acetylphenylhydrazine.

Fractions 11-12. These fractions were obtained as red amorphous powders.

The ether-benzene extracts (C) from the aqueous solution gave crystalline N-acetylphenylhydrazine after concentration of the solution. The crystals were filtered off, and further concentration of the filtrate yielded a dark brown syrup. This

was dissolved in benzene and adsorbed on a column of alumina (100g.). Benzene, or benzene:ether (1:1) did not elute anything.

Solvent	Fractions	Volume (ml.)	Weight (g.)
Ether	13	500	0.05
Ethanol:water	14	100	1.06
"	15	75	0.07

Fraction 14 gave a brown syrup which crystallised. Recrystallisation from benzene-light petroleum gave colourless plates of N-acetylphenylhydrazine, m.p. 128°C., not depressed by admixture with an authentic specimen.

The mother liquor, a brown syrup, failed to crystallise. Examination of this on a circular paper chromatogram showed that it contained glyoxalbisphenylhydrazone and glyserosazone.

Fraction 15 was obtained as a red amorphous powder after the solvent was removed. Hydrolysis in N-sulphuric acid, followed by paper chromatography, showed the presence of galactose and arabinose.

Separation of osazones by circular paper chromatograms.

Barry (66) found that sugarosazones can be rapidly separated

on a circular paper chromatogram. The fractions eluted from the alumina columns were examined by means of this technique.

Spots of a mixture of the osazones were made on a circle, 3 cm. in diameter, in the centre of a piece of Whatman No.1 filter paper (18.5cm. in diameter). This was placed between two glass plates and eluted with the solvent mixture toluene-ethanol-water (270:30:1) by means of a wick inserted into a slit in the centre of the paper (67,68). The glass plates with the chromatogram were placed over the container with the solvent so the wick was immersed in the solvent. A glass bell covered the plates and the container to avoid evaporation.

After two hours the osazones had separated into arcs which were easily visible.

There were used reference solutions of the osazones of glyoxal, glycerose, erythrose, arabinose and galactose.

Results:

Fraction 11, glycerosazone, and a very faint spot of galactosazone.

Fraction 12, glycerosazone, arabinosazone, galactosazone, and a red spot on the starting line.

Fraction 13, glyoxalbisphenylhydrazone.

Fraction 15, mainly arabinosazone, also some galactosazone together with faint zones of glyoxalbisphenylhydrazone, glycerosazone, and erythrosazone. There was also a red spot on the starting line.

Methylation of the Polysaccharides.

Methylation studies were carried out with different reagents on the polysaccharides. The methylated products were isolated at different stages and analysed for methoxyl content. Continuous treatment with dimethyl sulphate and sodium hydroxide (43) increased the methoxyl content slowly. This method was followed by Purdie's method (44) using methyl iodide and silver oxide. This proved, however, to be of little effect. More effective was the method using thallous ethoxide and methyl iodide (45).

Methylation of the undegraded polysaccharide.

The polysaccharide (25g.) was dissolved in water (100ml.) and methylated with dimethyl sulphate (285ml.) and sodium hydroxide (855ml.;30%). The reagents were added dropwise with vigorous stirring over a period of five hours. The reaction mixture was kept in ice under an atmosphere of nitrogen. It was stirred overnight, neutralised with dilute sulphuric acid and dialysed in a cellophane bag against tap water for six days. The aqueous solution was concentrated under reduced pressure, and afterwards extracted with chloroform. The partially methylated product was sparingly soluble in chloroform, so the methylation was continued.

This time the reagents were added in five batches over six days. One portion was added every day over five hours. At intervals acetone was added to keep the methylated product in solution.

After neutralisation, dialysis and concentration, the methylated product was extracted from the aqueous solution with chloroform, dried over anhydrous sodium sulphate and most of the chloroform distilled off under reduced pressure. The methylated product was obtained as a pale yellow hygroscopic powder by pouring the chloroform extract into an excess of light petroleum (b.p.60°-80°C.).

Yield = 19.5g. OMe = 41.5% $[\alpha]_D^{18}$-39.5°(c = 1.0 in chloroform).

Some of the partially methylated polysaccharide (9.5g.) was again methylated with dimethyl sulphate (275ml.) and sodium hydroxide (600ml.;30%), the reagents being added in four batches over five days. More and more acetone had to be added in order to keep the methylated product into solution. This gave a product having OMe = 43.1%.

The methylation was repeated, and the final product was a pale yellow glassy powder.

Yield = 5.3g. OMe = 44.9% $[\alpha]_D^{15}$-49.7°(c = 1.0 in chloroform)

The methoxyl content was not increased on further methylation.

A new quantity of ε-galactan (25g.) was given four series of five methylations. Dimethyl sulphate (75ml.) and sodium hydroxide (150ml.;30%) were added five times in each series, followed by neutralisation, dialysis (3 days) and concentration of the aqueous solution. The methoxyl content was constant after three series of five methylations had been carried out. Yield = 13.3g.

OMe = 44.2% $[\alpha]_D^{18}$-49.1° (c = 1.2 in chloroform).
$[\alpha]_D^{18}$-27.1° (c = 1.1 in methanol).

Methylation of the degraded polysaccharide.

In a similar experiment two series of five methylations were carried out on the degraded polysaccharide (10g.). Dimethyl sulphate (75ml.) and sodium hydroxide (150ml.;30%) were added five times in each series. After the last batch of reagents had been added to the reaction mixture, the partially methylated product precipitated as a brown sticky solid which was easily separated from the solution.

This solid contained inorganic material and was purified by repeated solution in chloroform followed by centrifuging and concentration. Several precipitations from light petroleum gave a pale yellow solid.

Yield = 8.2g. OMe = 39.5%.

Further methylation of the partially methylated product was carried out by means of thallous ethoxide and methyl iodide (45). The product was dissolved in benzene (100ml.) and a solution of thallous ethoxide (10g.) in chloroform (25ml.) was added. After shaking well, the chloroform was removed from the mixture by distillation under reduced pressure, the flask being protected from light during the process. The grey solid residue was finally powdered in the dark, and heated under reflux with methyl iodide (150ml.) until the yellow thallous iodide was no longer alkaline to phenolphthalein. The solvent was then filtered off, and the residue exhaustively extracted with boiling chloroform. All the filtrates were combined and dried over anhydrous sodium sulphate, concentrated, and the polysaccharide precipitated from light petroleum (b.p.60°-80°C.).

Yield = 6.2g. OMe = 42.1% $[\alpha]_D^{18}$-41.9°(c=0.9 in chloroform).

A new quantity of degraded ε-galactan (20g.) was given four series of five methylations, the procedure being the same as that described for undegraded polysaccharide. The methoxyl content was constant after three series of five methylations had been carried out. The fully methylated product was a pale yellow glassy solid.

Yield = 13.2g. OMe = 44.7% $[\alpha]_D^{18}$-49.8°(c=1.2 in chloroform).

Fractionation of the Methylated Polysaccharides.

Attempts were made to fractionate the methylated polysaccharides in order to obtain fractions of varying araban content. The solvents used were purified dry acetone, chloroform, and light petroleum (b.p.60°-80°C.).

The purification of the solvents was carried out as follows: Acetone was shaken with potassium permanganate, distilled, dried over potassium carbonate, filtered and redistilled. Chloroform was shaken with water, dried over anhydrous sodium sulphate, filtered and distilled. Light petroleum was shaken with concentrated sulphuric acid, followed by water, a saturated aqueous solution of sodium bicarbonate, and finally water. The solvent was dried over calcium chloride and distilled. The fraction (b.p.60°-65°C.) was used.

Fractionation of methylated ε-galactan with acetone and light petroleum.

Methylated ε-galactan (OMe = 41.5%, 4g.) was dissolved in acetone (20ml.) and light petroleum added dropwise from a burette to precipitate the methylated product. After the addition of 16ml. light petroleum a brown sticky precipitate had been formed. The supernatant liquid was decanted off, and the addition of

light petroleum to the solution continued (20ml.) till a new precipitate had been formed.

In this way there were obtained five fractions, all of which were hydrolysed in a sealed tube with N-sulphuric acid. The tubes were kept in the water-bath at 60°C. for two days, and then in boiling water (4 hours) to complete the hydrolysis.

The hydrolysis was followed by neutralisation of the solutions with barium carbonate, filtration and concentration. The respective syrups were examined on a paper chromatogram using the solvent (a).

The spots that appeared on the chromatogram had R_G values corresponding to the following sugars; tri-O-methyl-L-arabo-furanose, tetra-O-methyl-D-galactose, di- and tri-O-methyl-L-arabinose, tri-O-methyl-D-galactose, di- and mono-O-methyl-D-galactose, and also a long cherry red streak just below the starting line. The colour and the shape of the cherry red spot were similar to those of a uronic acid. The colour of this spot was strongest for the first fractions.

The intensity of the spots of methylated arabinose was the same for all fractions.

From the chromatographic examination it seemed as if no significant fractionation had taken place of the methylated polysaccharide, except that the three first fractions contained more uronic acid than fractions number four and five.

Fractionation of methylated ε-galactan with light petroleum and chloroform.

The methylated ε-galactan (7.0g., OMe = 41.5%) was fractionated by the solution method, the solvent being a mixture of light petroleum and chloroform. The amount of chloroform in the extraction mixture was increased stepwise. The methylated product was heated with the solvent mixture under reflux for two hours. The flask was then cooled, and when the solid had settled completely the supernatant liquid was decanted off.

The fractionation experiment using light petroleum/acetone showed that paper chromatography only could not give definite information as to whether an araban-rich fraction had been obtained or not. In the following experiments the amount of araban (calculated as dimethyl araban) was estimated by distillation with hydrochloric acid and precipitation of furfural with phloroglucinol (the procedure will be described in a following chapter).

The results from the fractionation are given below. The optical rotations were observed in chloroform, c = 1, the temperature varying between 16°-22°C.

Fraction	Solvent(100ml.) petrol.:chlorof.	Weight (g.)	$-OCH_3$ %	$[\alpha]_D$	Dimethyl araban %
F I	100	0.00			
F II	95:5	0.01			
F III	90:10	0.09			
F IV	80:20	2.31	44.3	-38.8	5.6
F V	70:30	3.65	42.1	-39.2	5.4 (F V A)
					4.8 (F V B)
F VI	Left from F V	0.92	40.5	-39.5	4.6

Fraction F V (soluble in 70:30 petroleum-chloroform) was fractionated further by solving it in chloroform (25ml.) followed by the addition of light petroleum (75ml.). Some brown syrup, F V B, was precipitated (1.18g.). The liquid was decanted off and evaporated to dryness under diminished pressure to give a white glassy solid F V A. The following analytical data were found:

F V A OMe = 44.1% $[\alpha]_D$-39.9° dimethylaraban = 5.4%.

F V B OMe = 40.3% $[\alpha]_D$-39.6° dimethylaraban = 4.8%.

The fractions were hydrolysed and examined on a paper chromatogram. The result was identical with that from the first fractionation. F V A, F V B and F VI contained more uronic acid than the first fractions.

Fractionation of fully methylated ε-galactan.

The partially methylated polysaccharide was methylated further. The fully methylated product (4.0g.,OMe=44.9) was fractionated by the solution method just described.

Fraction	Solvent(100ml.) petrol.:chlorof.	Weight (g.)	$-OCH_3$ %	$[\alpha]_D^{16°}$
f I	100:0	0.00		
f II	90:10	0.43	44.8	-49.9
f III	85:15	3.45	44.6	-49.7

Hydrolysis followed by paper chromatography gave no new information.

The fully methylated polysaccharide, which had been achieved after four series of five methylations had been carried out, was fractionated by the solution method. This methylated product (10g.) had OMe = 44.2%, $[\alpha]_D^{18}$-49.1°(c = 1.2 in chloroform) and contained 5.2% dimethyl araban.

Fraction	Solvent(100ml.) petrol.:chlorof.	Weight (g.)	$-OCH_3$ %	Dimethyl araban %
Fa I	85:15	0.75	44.2	5.1
FaII	80:20	9.05	44.1	

Since the methoxyl content and the amount of dimethyl araban in the first small fraction Fa I are identical with those found in the original methylated polysaccharide, no fractionation had taken place.

Fractionation of methylated degraded ε-galactan.

Partially methylated degraded ε-galactan (6.0g., OMe=42.1%) was fractionated by the solution method just described for the undegraded product.

Fraction	Solvent(100ml.) petrol.:chlorof.	Weight (g.)	$-OCH_3$ %	$[\alpha]_D$	Dimethyl araban %
F D I	100:0	0.01			
F D II	95:5	0.02			
F D III	90:10	0.03			
F D IV	80:20	2.00	43.5	-42.1	3.9
F D V	75.25	3.60	41.8	-40.6	3.6
F D VI	70:30	brown sticky solid			

F D V was, after further methylation with thallous ethoxide and methyl iodide, soluble in 80:20 petroleum-chloroform. OMe = 44.2%, $[\alpha]_D^{21}$ -46.9 (c = 1.0 in chloroform).

Hydrolysis followed by paper chromatography indicated that F D V contained more uronic acid than F D IV. F D V was not hydrolysed quite so readily as F D IV.

Fractionation of fully methylated degraded ε-galactan.

Complete methylation had been achieved by giving the degraded polysaccharide four series of five methylations. This fully methylated product (10g.) was fractionated after the solution method. OMe = 44.7%, $[\alpha]_D^{18}$ -49.8°, dimethyl araban = 4.1%.

Fraction	Solvent(100ml.) petrol.:chlorof.	Weight (g.)	$-OCH_3$ %	Dimethyl araban %
Fa D I	85:15	0.80	44.7	3.8
Fa D II	80:20	9.15	44.8	4.0

Some brown sticky solid was left in the flask.

Estimation of Araban by Distillation with Aqueous Hydrochloric Acid.

The conversion of unsubstituted pentoses into furfural by the action of boiling aqueous hydrochloric acid has been used as

the basis of several methods of estimation of these substances. Bott and Hirst (46) have shown that also the fully methylated derivatives of arabofuranose, arabopyranose, xylofuranose, and xylopyranose are readily decomposed by hydrochloric acid with formation of furfural, the rates of reaction and the amounts of furfural produced being directly comparable with those found with the free sugars.

In the present work it was of interest to estimate the amount of araban in the different fractions obtained after the methylated polysaccharides had been fractionated with light petroleum-chloroform. There was used a standard method for the estimation of furfural by means of phloroglucinol (47). Because only relatively small amounts of methylated polysaccharide were available, the method was first tried out on 30mg. methylated xylan which theoretically would produce the same amount of furfural as 500mg. methylated ε-galactan. One experiment was also carried out on the original polysaccharide.

The procedure was as follows: The sample was placed in a flask (250ml.) provided with a separating funnel, and also with a trap at the outlet tube. Hydrochloric acid (12% exactly)(100ml.) was carefully added to avoid the polysaccharide from sticking to the sides of the flask. Glass beads were used to avoid bumping. The flask was kept in an oil-bath (180°C.) so the liquid distilled at the rate of 30ml. in ten minutes. As soon as 30ml. distillate

had been collected, the same amount of hydrochloric acid (12%) was added to the flask from the separating funnel. The distillation was continued in this manner until the distillate was free from furfural. One drop of the distillate was tested for furfural on a filter paper with a drop of aniline reagent (equal amounts of aniline and water made to a clear solution with hydrochloric acid). As long as any furfural was present the distillate would give a pink colour when tested.

In the case of methylated xylan it was sufficient to collect 320ml. distillate to which 30ml. phloroglucinol (0.8% in 12% hydrochloric acid $^{W}/_{v}$) was added, and the distillate made up to 400 ml. with 12% hydrochloric acid.

In the case of methylated ε-galactan there was collected 700ml. distillate which was collected in two batches. To both of them was added an equal amount of phloroglucinol solution (30ml.), and each distillate made up to 400ml. After complete precipitation (42 hours) the solutions were filtered through a tared filter crucible, Gooch 4, the precipitate washed with water (150ml.) and dried at 100°-105°(4 hours).

The crucible was then placed in a narrow beaker with 95% alcohol (20ml.) and heated at 60°C. in a water-bath (10 minutes). The alcohol was removed at the pump, the crucible dried at 100°C. and weighed.

Under standard conditions, the volume of distillate collected is 400ml., and the weight of pentosan is obtained from the weight of phloroglucide by the formula P=(a+0.0052)x0.8949, in which P is the weight of pentosan, a that of the phloroglucide compound, and 0.0052 is a correction factor to compensate for the solubility of the latter. In the case of the methylated ε-galactan (undegraded and degraded) this factor was proportionately increased.

Results:

ε-Galactan.

wt. of substance = 459.58mg.
wt. of phloroglucide = 49.63mg.

Araban = 10.7%

Methylated Xylan.

Wt. (mg.)	Wt. (mg.) phloroglucide	Recovery % (dimethyl xylan)
26.74	19.60	100.6
35.49	28.42	101.2

Methylated ε-galactan (undegraded and degraded).

	Wt.(mg.)	Wt.(mg.) phloroglucide	Dimethyl araban %
F IV	504.22	14.83	5.4
F IV	450.38	13.81	5.8
F V A	382.40	10.03	5.4
F V B	506.81	11.91	4.8
F VI	454.83	8.85	4.6
F a	459.68	11.54	5.2
F a I	569.70	16.37	5.1
F D IV	504.30	7.90	3.9
F D V	522.83	7.15	3.6
F a D	573.40	11.75	4.1
F a D I	621.64	11.30	3.8
F a D II	551.50	9.71	4.0

F a = methylated ε-galactan before fractionation.

F a D = methylated degraded ε-galactan before fractionation.

According to Bott and Hirst (46) the transformation of trimethyl arabopyranose is only 66% when boiled with 20%

hydrochloric acid. The rate of decomposition is also slower for this sugar than it is for trimethyl arabofuranose and trimethyl xylopyranose.

These facts were not taken into consideration in the experiments just described. The amount of araban, dimethyl xylan, and dimethyl araban was estimated from the formula mentioned, and the only correction made was to increase the correction factor (0.0052) in those cases where the distillate exceeded 400ml.

However, the estimations of dimethyl araban made it possible to compare the different fractions, and showed that no significant fractionation into an arabinose-rich part had taken place.

Hydrolysis of the Methylated Polysaccharides.

The methylated polysaccharide (10 g.) was suspended in 2N-sulphuric acid (150 ml.) and left at room temperature till the polysaccharide had dissolved. The mixture was diluted with distilled water (150 ml.) and heated under reflux on the water-bath. The temperature was slowly increased, so the methylated polysaccharide always was kept in solution. Finally, after two days the hydrolysis was completed at 100°C. for seven hours.

The acid solution was neutralised with barium carbonate, the precipitate removed by filtration and washed exhaustively with water. The combined filtrates were concentrated under reduced pressure. When about 50 ml. remained in the flask, the solution was filtered through Whatman No.42 filter paper. Further concentration of the filtrate yielded a pale yellow clear syrup which was dried *in vacuo* over phosphoric oxide. Yield = 9.8 g.

Separation of the Methylated Sugars on a Cellulose Column.

The syrupy mixture of methylated sugars obtained after hydrolysis of the methylated polysaccharide was fractionated on a column of cellulose powder. The column (3.5 cm. x 80 cm.) was made according to the method of Hough, Jones, and Wadman(49).

The solvents used for eluting the column were purified light petroleum (b.p. 100°-120°C.), and purified n-butanol. The butanol was purified by boiling it (1 l.) under reflux over sodium hydroxide pellets (10 g.) for four hours. Direct distillation yielded a pure butanol.

Before use the column was washed with water (1.5 l.), butanol half saturated with water (1 l.), and finally with light petroleum/butanol (70/30, 1 l.) saturated with water.

The sugars were eluted from the column with petroleum/butanol (70/30) saturated with water, the proportion of butanol in the mixture being gradually increased as the sugars were removed. Finally, after all the methylated sugars had come through the column, the solvent was changed to water to wash out unmethylated sugars and uronic acids.

The sugar syrup (5 g.) was dissolved in a minimum of the solvent (70/30), added dropwise to the top of the column, and allowed to soak in. A thin layer of cellulose powder and of cotton wool was then placed on the top of the column and 100 ml. of the solvent added. The column was left for three hours.

A reservoir containing petroleum/butanol (70/30) saturated with water was inverted on the top of the column, and the eluate collected on the automatic turntable in tubes containing 6 ml. each.

Every tenth tube was tested for sugars. The tube was kept in hot water and the solvent removed with compressed air. The remaining syrup was spotted on a paper chromatogram and examined for sugars.

A complete separation between the successive compounds was not achieved, especially not for the higher methylated sugars. The tubes which contained the same sugar, or the same sugar mixture were combined, and the solvent removed under

reduced pressure. The resultant syrup was dissolved in water, treated with charcoal, filtered, and evaporated to dryness *in vacuo* over phosphoric oxide.

The results from the paper chromatograms using solvent (b) are tabulated below.

Undegraded Polysaccharide.

Fraction	Weight (g.)	Colour	R_G Value	Possible Sugar
a	0.0057	grey	0.96	2:3:5-trimethyl-arabinose
b	0.4372	grey red/brown	0.96 0.90	2:3:5-trimethyl-arabinose (trace) 2:3:4:6-tetramethyl-galactose
c	0.7007	red/brown	0.90	2:3:4:6-tetramethyl-galactose
d	0.0331	red/brown grey pink	0.90 0.86 0.83	2:3:4:6-tetramethyl-galactose 2:5-dimethyl- and 2:3:4-trimethyl-arabinose
e	0.2111	grey pink	0.86 0.83	2:5-dimethyl- and 2:3:4-trimethyl-arabinose
f	0.0218	grey pink red/brown	0.86 0.83 0.72	as for *e* 2:3:4- and 2:4:6-trimethyl-galactose
g	0.9699	red/brown	0.72	2:3:4- and 2:4:6-trimethyl-galactose
h	0.0573	red/brown grey,pink brown	0.72 0.59 0.52	as for *g* ? ?
i	1.3147	brown	0.46	2:4-dimethyl-galactose
j	0.2598	brown	0.30	2-methyl-galactose
k	0.1009	brown,pink cherry streak		galactose, arabinose, methylated uronic acid

Total = 4.1122 g.

Degraded Polysaccharide.

Fraction	Weight (g.)	Colour	R_G Value	Possible Sugar
A	0.5668	grey	0.96	2:3:5-trimethyl-arabinose
		red/brown	0.90	2:3:4:6-tetramethyl-galactose
B	0.7400	red/brown	0.90	2:3:4:6-tetramethyl-galactose
C	0.0485	red/brown	0.90	as for B
		pink	0.83	2:3:4-trimethyl-arabinose
D	0.1935	grey	0.86	2:5-dimethyl- and
		pink	0.83	2:3:4-trimethyl-arabinose
F	1.0220	red/brown	0.72	2:3:4- and 2:4:6-trimethyl-galactose
G	0.0100	red/brown	0.72	as for F
		pink	0.68	?
		grey/brown	0.59	?
H	0.0653	red/brown	0.72	as for F
		grey-brown	0.59	?
		red/brown	0.52	?
I	0.0787	red/brown	0.72	as for F
		red/brown	0.48	?
K	1.1472	brown	0.46	2:4-dimethyl-galactose
L	0.0221	pink	0.67	?
		pink	0.60	?
		brown	0.46	as for K (mainly)
M	0.0318	brown	0.46	as for K
		brown	0.30	2-methyl-galactose (mainly)
N	0.1387	brown	0.30	2-methyl-galactose
O	0.0497	brown	0.30	2-methyl-galactose
		pink	0.18	arabinose
P	0.0922	pink, brown cherry streak		arabinose, galactose, methylated uronic acid

Total = 4.2065 g.

According to the weights of the fractions obtained from the cellulose columns, the molecular proportions of the methylated sugars are as follows:

	Undegraded polysacch.	Degraded polysacch.
Tetramethyl-galactose	5.0 moles	5.7 moles
Trimethyl-galactose	4.4 "	4.9 "
Dimethyl-galactose	5.8 "	5.2 "
Monomethyl-galactose	1.3 "	1.1 "
Di- and trimethyl-arabinose	1.2 "	1.1 "
Methylated uronic acid	ca. 2%	ca. 2%

Examination of Fractions.

Undegraded Polysaccharide.

Fraction a. 2:3:5-Tri-O-methyl-L-arabinose.

This fraction was obtained as a syrup but was too small to be identified except on the paper chromatogram. It gave a grey spot which had the same R_G value and colour as an authentic specimen of 2:3:5-tri-O-methyl-L-arabinose. The

grey spot gave a pink fluorescence in ultraviolet light.

Demethylation with hydrobromic acid (51).

The syrup (5 mg.) was demethylated with hydrobromic acid (1 ml., 48% w/w) by heating at 100°C. in a sealed tube for 10 minutes. The tube was opened and the content immediately diluted to 10 ml. with water. The mixture was neutralised with silver carbonate, filtered, and dissolved silver removed with hydrogen sulphide gas. After filtration through a bed of charcoal, the solution was concentrated under reduced pressure. Examination on a paper chromatogram, using a reference mixture of arabinose and galactose, showed the presence of arabinose, trace of galactose, and some faster travelling pink spots of partially methylated arabinose.

Fraction c. 2:3:4:6-Tetra-O-methyl-D-galactose.

This fraction crystallised on keeping, and was recrystallised from ether-light petroleum to yield crystals m.p. 68°C.

OMe = 53.1% (calculated for $C_{10}H_{20}O_6$, 52.5%).
$[\alpha]_D^{19}$ + 142°(5 min.), 130°(30 min.), 119°(1 hr.), 117°(3 hrs., constant) (c = 1.1 in water).

Preparation of anilide.

The syrup (50 mg.) was treated with a solution of freshly distilled aniline (50 mg.) in ethanol (2 ml.). The mixture was heated under reflux on the boiling water-bath (30 minutes). On cooling, a crystalline solid separated. Recrystallisation from ethanol yielded crystals m.p. 192°C., not depressed by admixture with an authentic specimen of 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine.

Fraction b. 2:3:5-Tri-O-methyl-L-arabinose and 2:3:4:6-tetra-O-methyl-D-galactose.

Paper chromatography of this fraction showed it was a mixture of 2:3:5-tri-O-methyl-L-arabinose and 2:3:4:6-tetra-O-methyl-D-galactose.

Demethylation with hydrobromic acid followed by paper chromatography showed the presence of arabinose and galactose; galactose appeared to be in greatest amount.

OMe = 52.5% (calculated for $C_{10}H_{20}O_6$, 52.5%) found on analysis indicated that very little of the methylated pentose was present.

The syrup crystallised on inoculation with a crystal of 2:3:4:6-tetra-O-methyl-D-galactose. The crystals were dried on a tile, and recrystallisation from ether-light petroleum gave crystals m.p. 68°C.

Treatment with alcoholic aniline gave 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 192°C.

Fraction g. Tri-O-methyl-D-galactose.

This fraction crystallised on keeping. The crude crystals were dried in vacuo at 60°C. over phosphoric oxide for 2 hours and had OMe = 41.3% (calc. for $C_9H_{18}O_6$, 41.9%).

Demethylation with hydrobromic acid followed by paper chromatography showed that galactose was the only free sugar present.

Optical rotation.

Without drying, $[\alpha]_D^{20}$ + 125° (5 min.), 115° (30 min.), 112° (1 hr.), 104° (2 hrs. constant) (c = 1.1 in water).

After drying in vacuo at 60° C., $[\alpha]_D^{20}$ + 109° (constant).

Recrystallisation.

The crude crystals were recrystallised from acetone-ether-light petroleum and gave long white fibres which melted at 56°C. Further recrystallisation did not alter the melting point.

2:3:4-Tri-O-methyl-α-D-galactose monohydrate is reported

(53,50) to have a melting point 75°-80°C. and 2:4:6:tri-O-methyl-α-D-galactose 102°-105°C.(54).

Methoxyl found on analysis without drying was

OMe = 38.2% (calc. for $C_9H_{18}O_6 \cdot H_2O$, 38.7%).

Optical rotation.

$[\alpha]_D^{20}$ + 139° (5 min.), 128° (30 min.), 117° (1 hr.), 109° (2 hrs., constant). (c = 1.1 in water).

The optical rotation for 2:3:4-tri-O-methyl-α-D-galactose monohydrate is reported (50) to be + 152° → + 114°, and for 2:4:6-tri-O-methyl-α-D-galactose (54) + 124° → + 90.4°.

Preparation of anilide (48).

A sample (100 mg.) freed from moisture by heating it at 60°C. in vacuo was treated with aniline (70 mg.) and alcohol (5 ml.) and heated under reflux at 100°C. for 5 hours. The bulk of the solvent was removed by distillation under reduced pressure and the remaining syrup kept at 0°C. overnight. The crystals were filtered off, and recrystallisation from acetone yielded the plate shaped crystals of 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine m.p. 166°C., not depressed by admixture with an authentic specimen. In admixture with 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine (m.p. 171°C) the melting point was depressed to 144°C.

The mother-liquor crystallised further when kept at 0°C., and gave a mixture of plates and needles which had m.p. 143°C. The needles were probably 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine. It was not succeeded to separate the two crystalline products either by recrystallisation from acetone or by flotation (the anilide of 2:3:4-tri-O-methyl-D-galactose is the denser of the two (57)).

Fraction i. 2:4-Di-O-methyl-D-galactose.

The colourless syrup crystallised completely, and was recrystallised from acetone containing 1% of water. The monohydrate thus obtained had m.p. 102°C.

OMe = 27.1% (calculated for $C_8H_{16}O_6 \cdot H_2O$, 27.2%).

Optical rotation.

$[\alpha]_D^{20}$ + 136° (5 min.), 124° (15 min.), 113° (45 min.), 86° (2 hrs., constant), (c = 1.1 in water).

Preparation of anilide.

A sample of the fraction (50 mg.) was heated with aniline (50 mg.) in alcohol (2 ml.) under reflux on the boiling water-bath for one hour. At the end of this time crystals separated from the hot solution. After cooling, the crystals were

removed by filtration. Recrystallisation from methyl alcohol yielded 2:4-di-O-methyl-N-phenyl-D-galactosylamine m.p. 215°C. (decomp.) (50) not depressed by admixture with an authentic specimen.

Fraction j. 2-O-methyl-D-galactose.

This fraction crystallised in large crystals. The crude crystals had OMe = 16.1% (calc. for $C_7H_{14}O_6$, 16.8%) which showed that it was a monomethyl hexose.

Examination on a paper chromatogram in the solvent (a) using a reference mixture of ribose, arabinose, and galactose showed a brown spot which travelled slightly faster than ribose. Both 4-O-methyl- and 6-O-methyl-D-galactose would travel slower than ribose (52).

Recrystallisation from glacial acetic acid yielded crystals m.p. 148°-149°C., not depressed by admixture with an authentic specimen of 2-O-methyl-D-galactose.

Optical rotation.

$[\alpha]_D^{20}$ + 53° (5 min.), 68° (30 min.), 80° (1 hr.),
+ 85° (2 hrs., constant), (c = 0.9 in water).

Preparation of anilide.

A sample (30 mg.) was heated with aniline (30 mg.) in

alcohol (2 ml.) under reflux on the boiling water-bath for 1.5 hours. The bulk of the solvent was removed by distillation. On addition of light petroleum to the remaining solution the anilide crystallised. The crystals were removed by filtration and recrystallised from ether-light petroleum to yield 2-O-methyl-N-phenyl-D-galactosylamine, m.p.163°C.

Fraction e. 2:3:4-Tri-O-methyl-and 2:5-di-O-methyl-L-arabinose.

This fraction was obtained as a syrup.

OMe = 41.0%, $[\alpha]_D^{20}$ + 82° (c = 1.2 in water).

The syrup gave two spots on the paper chromatogram, one grey (R_G 0.86), and one pink (R_G 0.83) using the solvent (b). The grey spot had a pink fluorescence in ultraviolet light.

An authentic specimen of 2:3:4-tri-O-methyl-L-arabinose gave a pink spot (R_G 0.83) on the paper chromatogram. An authentic specimen of 2:5-di-O-methyl-L-arabinose gave a grey spot (R_G 0.86) which had a pink fluorescence in ultraviolet light. 3:5-Di-O-methyl-L-arabinose gave a brown spot which was yellow in ultraviolet light.

When the solvent benzene-ethanol-water was used, the grey spot would travel slower than the pink.

Demethylation followed by paper chromatography (solvent a,b) gave arabinose together with some faster moving pink spots, the R_G values of which corresponded to those of 2-O-methyl-L-arabinose,

3:4-di-O-methyl-, 2:4-di-O-methyl-, and 2:3-di-O-methyl-L-arabinose (11,52).

Separation on a charcoal-Celite column.

An attempt was made to separate the mixture on a charcoal-Celite column according to the method of Lindberg and Wickberg (55) using linear gradient elution with aqueous ethyl methyl ketone.

Equal parts of charcoal and Celite were mixed and treated with conc. hydrochloric acid. The mixture was thoroughly washed with water, ethanol, and finally with 2.5% aqueous ethyl methyl ketone, and poured into the column (1.5 cm. x 50 cm.) as a thick slurry.

The sugar syrup (92 mg.) was dissolved in 2.5% ethyl methyl ketone (2 ml.) and added to the top of the column. The eluant was taken from two flasks with the same diameter, containing 500 ml. each of 2.5% and of 5.5% aqueous ethyl methyl ketone. The fractions (1.5 ml. each) were collected in tubes on the turntable. Every tenth tube was evaporated to dryness and examined on a paper chromatogram using the solvent (d). The tubes containing the same sugars were collected and the solvent distilled off under reduced pressure.

Distribution of the sugars.

Tubes	Identity	Weight
10-40	Nothing	
50-66	Mixture of trimethyl- and dimethyl-arabinose	44.0 mg.
67-70	Dimethyl-arabinose	11.3 mg.

Separation using thick paper chromatograms.

The syrupy mixture (44 mg.) from the charcoal-Celite experiment was spotted on a thick paper chromatogram (Whatman No.1, 3 mm.) and run in solvent (d). The side strips were cut off and sprayed to localise the respective sugars. A brown spot on the starting line indicated that some methylated galactose had been present in the syrup.

The zones containing methylated arabinose were cut out and extracted with cold water to give 2:3:4-tri-O-methyl-L-arabinose (11.3 mg.) and 2:5-tri-O-methyl-L-arabinose (20.1 mg.) after the water had been removed *in vacuo*.

A syrupy mixture of the two methylated pentoses (270 mg.) obtained from another cellulose column experiment was separated on thick paper chromatograms by the method just described. The water was removed *in vacuo* over phosphoric oxide to give colourless syrups of tri-O-methyl-L-arabinose (111 mg.) and of di-O-methyl-L-arabinose (154 mg.). A complete separation of

the two sugars was not obtained which was shown by paper chromatography.

Tri-O-methyl-L-arabinose fraction.

This fraction was obtained as a colourless syrup with $[\alpha]_D^{20}$ + 120° (c = 2.2 in water) and OMe = 46.1% (calculated for $C_8H_{16}O_5$, 48.4%).

Demethylation with hydrobromic acid and examination on the paper chromatogram (solvent a) gave spots of arabinose, trace of galactose, and some pink spots. The pink spots had R_G values corresponding to di-O-methyl-L-arabopyranose.

Preparation of phenylhydrazide (74).

The syrup (100 mg.) was oxidised with bromine water at room temperature for four days. Excess of bromine was removed by aeration, and the solution neutralised with silver carbonate. After filtration the arabonic acid was liberated with hydrogen sulphide gas, and the solution boiled for a few minutes with a pinch of charcoal. The filtered, clear solution was evaporated to a syrup under reduced pressure. The syrup was heated *in vacuo* at 90°C. for one hour. The phenylhydrazide of 2:3:4-tri-O-methyl-L-arabonic acid was prepared by treating the lactone with phenylhydrazine (150 mg.) first in boiling dry methanol-ether and then, after removal of the solvent, for

3 hours at 90°C. The crystalline product of the phenylhydrazide of 2:3:4-tri-O-methyl-L-arabonic acid after recrystallisation from alcohol-ether-light petroleum had m.p. 157°C.

Di-O-methyl-L-arabinose fraction.

This fraction was obtained as a colourless syrup which failed to crystallise. Paper chromatography showed that it was admixed with 2:3:4-tri-O-methyl-L-arabinose.

The methoxyl content was OMe = 33.9% (calculated for $C_7H_{14}O_5$, 34.8%), and the optical rotation was $[\alpha]_D^{18}$ -3° (c = 1.5 in water).

Demethylation followed by paper chromatography (solvent a) gave spots of arabinose, trace of galactose, and one pink spot with R_G value similar to that of 2:4-di-O-methyl-D-galactose. This might be 2-O-methyl-L-arabinose(52).

Preparation of amide (74).

The syrup (100 mg.) was treated with bromine water at room temperature for 3 days. The excess of bromine was removed by aeration, and the solution neutralised with silver carbonate. After treatment with hydrogen sulphide gas, the filtered solution was concentrated to a syrup under reduced pressure. The resultant syrup was heated at 90°C. in vacuo for 2 hours. The crude lactone thus obtained was treated with methanolic ammonia

at 0°C. for 48 hours. Removal of the solvent gave a syrup which crystallised on keeping. The 2:5-di-O-methyl-L-arabonamide had m.p. 131°C. after recrystallisation from alcohol-ether.

Fraction d and Fraction f.

Paper chromatography showed that Fraction d was a mixture of tetra-O-methyl-D-galactose, tri-O-methyl-, and di-O-methyl-L-arabinose. Fraction f was a mixture of tri-O-methyl-D-galactose, and the two methylated pentoses just mentioned. Demethylation with hydrobromic acid followed by paper chromatography showed the presence of galactose and arabinose.

Separation of sugars using thick paper chromatograms.

The two fractions d and f were combined (41 mg.) and spotted on thick paper chromatograms (Whatman No.1, 3 mm.) prepared for quantitative work; benzene-ethanol-water solvent was used. The sugars were localised by spraying the side strips with aniline oxalate and were extracted from the corresponding sugar zones with cold water.

Tri-O-methyl-L-arabinose	=	11.2 mg.
Di-O-methyl-L-arabinose	=	21.6 mg.

Fraction h.

This fraction on a paper chromatogram gave the spots of

tri-O-methyl-D-galactose together with some slower travelling spots. Demethylation with hydrobromic acid followed by examination on a paper chromatogram gave a strong spot of galactose and a faint arabinose spot.

Fraction k. Free sugars and methylated uronic acid.

The yellow syrup was dissolved in water and treated with resin Amberlite IR 120 to remove barium ions. The resin was filtered off and the filtrate evaporated to dryness under reduced pressure.

When the syrup was examined on the paper chromatogram using solvent (e) there appeared a cherry spot (R_G 0.84) the colour and shape of which were similar to those of a methylated uronic acid (56). Galactose and arabinose were also present but in smaller quantities.

Preparation of 2:3:4-tri-O-methyl-L-arabinose.

β-Methyl-arabopyranoside was made according to the method of Hudson (69). Arabinose (10 g.) was heated with dry methanolic hydrogen chloride (100 ml.; 1.5%) under reflux on the boiling water-bath (3 hrs.). The solution was neutralised by silver carbonate in the usual way and concentrated under reduced pressure. On standing, β-methyl-L-arabopyranoside crystallised, and was removed by filtration. The crystals were

freed from the adhering syrup by extraction with ethyl acetate and recrystallisation from alcohol. The product melted at 169°C. and had $[\alpha]_D^{19}$ + 245° (c = 0.8 in water).

β-Methyl-L-arabopyranoside was dissolved in water and given two series of two methylations with sodium hydroxide and dimethyl sulphate (70). Each series was followed by extraction of the aqueous solution with chloroform and removal of the chloroform (after drying) under reduced pressure. Removal of the chloroform after the second series of methylations gave a pale yellow mobile syrup which gave crystalline β-methyl-2:3:4-tri-O-methyl-L-arabopyranoside when kept *in vacuo* over phosphoric oxide. This compound is soluble in all ordinary organic solvents (70) and is difficult to recrystallise. The crude crystals were hydrolysed with N-sulphuric acid and neutralised with barium carbonate in the usual way. Removal of the water under reduced pressure gave a colourless syrup, OMe = 47.2%, $[\alpha]_D^{20}$ + 128° (c = 0.9 in water). Examination on the paper chromatogram in solvent (b) gave a distinct red spot (R_G 0.83) of 2:3:4-tri-O-methyl-L-arabinose together with some very faint slower travelling pink spots (caused by under-methylation).

Degraded Polysaccharide.

Fraction A. 2:3:5-Tri-O-methyl-L-arabinose and 2:3:4:6-tetra-O-methyl-D-galactose.

Paper chromatography showed that this fraction was a mixture

of the two sugars mentioned above. Demethylation and examination on a paper chromatogram gave spots of arabinose and galactose.

The colourless syrup crystallised after it had been inoculated with a crystal of 2:3:4:6-tetra-O-methyl-D-galactose. The crystals were removed from the adhering syrup by tiling and recrystallised from ether-light petroleum to yield crystals m.p. 69°C.

The crystals (50 mg.) were treated with alcoholic aniline, the procedure being the same as for the undegraded polysaccharide. 2:3:4:6-Tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 192°C. was obtained, showing no depression in melting-point by admixture with 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine from the undegraded product.

The syrup gave on analysis OMe = 52.0% which indicated that very little of the methylated pentose was present. Tetra-O-methyl-hexose requires OMe = 52.5%, and tri-O-methyl-pentose requires OMe = 48.4%.

Fraction B. 2:3:4:6-Tetra-O-methyl-D-galactose.

This fraction crystallised and was recrystallised from ether-light petroleum. The crystals melted at 69°C. OMe = 52.8%.

Before the optical rotation was measured the crystals were dried *in vacuo* at 60°C. over phosphoric oxide (1.5 hours).

$[\alpha]_D^{19}$ + 117° (constant), (c = 1.5 in water).

The anilide formation was carried out in the usual way, and 2:3:4:6-tetra-O-methyl-N-phenyl-D-galactosylamine m.p. 192°C. was obtained in good yield.

Fraction C. 2:3:4:6-Tetra-O-methyl-D-galactose and 2:3:4-tri-O-methyl-L-arabinose.

Paper chromatography showed that this fraction was a mixture of these two sugars. Demethylation and examination on a paper chromatogram showed galactose (in quantity) and arabinose.

The syrup (43 mg.) was separated on a thick paper chromatogram (Whatman No.1, 3 mm.) using the solvent (d). Cold water extraction of the zone containing tri-O-methyl-L-arabinose, gave after the solvent had been removed 10.4 mg. of this sugar. This indicated that three-quarters of this fraction consisted of 2:3:4:6-tetra-O-methyl-D-galactose.

Fraction D. 2:3:4-Tri-O-methyl-L-arabinose and 2:5-di-O-methyl-L-arabinose.

This fraction was obtained as a colourless syrup which failed to crystallise.

OMe = 40.2%, $[\alpha]_D^{18}$ + 65° (c = 1.0 in water).

Paper chromatography showed that the syrup was a mixture of two substances, one giving a pink spot (R_G 0.83) and the other a grey spot (R_G 0.86) using the solvent (b).

The syrup (147 mg.) was separated on thick paper chromatograms (Whatman No.1, 3 mm.) using the solvent (d). On heavily spotting of the papers it appeared that the syrup contained some 2:3:4:6-tetra-O-methyl-D-galactose. The paper strips containing the respective sugars were extracted with cold water (3 ml.) The water was removed *in vacuo* over phosphoric oxide. A complete separation of the two sugars was not achieved which was shown afterwards by paper chromatography.

In some cases streaking on the chromatogram caused that it was impossible to separate tetra-O-methyl-D-galactose and tri-O-methyl-L-arabinose completely. Some of the tri-O-methyl-L-arabinose was therefore lost.

Tri-O-methyl-L-arabinose fraction (66.5 mg.) $[\alpha]_D^{20}$ + 118° (*c* = 0.7 in water), OMe = 46.8%.

Di-O-methyl-L-arabinose fraction (74.3 mg.), $[\alpha]_D^{20}$ - 9° (*c* = 0.7 in water), OMe = 33.8%.

Fraction G.

Paper chromatography showed that this fraction contained tri-O-methyl-D-galactose together with two slower travelling sugars. Demethylation and examination on a paper chromatogram gave spots of arabinose and galactose.

Fraction H, and Fraction I.

These two fractions contained tri-O-methyl-D-galactose

together with two other components which travelled slower on a paper chromatogram. Demethylation followed by paper chromatography showed the presence of galactose only.

Fraction K. 2:4-Di-O-methyl-D-galactose.

This fraction crystallised when it was kept in vacuo over phosphoric oxide. Recrystallisation from acetone containing 1% water gave the monohydrate, m.p. 102°C., not depressed by admixture with an authentic specimen of 2:4-di-O-methyl-D-galactose monohydrate.

OMe = 29.9%.

$[\alpha]_D^{18}$ + 133° (5 min.), 121°(15 min.), 98° (1 hr.), 86° (2 hrs., constant) (c = 1.0 in water).

The anilide was prepared as previously described, m.p. 215°C. (decomp.) not depressed by admixture with 2:4-di-O-methyl-N-phenyl-D-galactosylamine from undegraded product.

Fraction L.

Paper chromatography showed that this fraction contained 2:4-di-O-methyl-D-galactose (mainly) together with some faster travelling substances. Demethylation followed by examination on a paper chromatogram gave galactose (in quantity) and arabinose.

Fraction N. 2-O-Methyl-D-galactose.

This fraction crystallised and contained OMe = 16.3%. The

optical rotation was

$[\alpha]_D^{19}$ + 51° (5 min.), 58° (30 min.), 70° (1 hr.), 81° (2 hrs.), 84° (3 hrs., constant), (*c* = 1.1 in water).

The product was recrystallised from glacial acetic acid, washed with alcohol and gave crystals m.p. 148°-151°C., not depressed by admixture with an authentic specimen of 2-O-methyl-D-galactose (m.p. 149°-153°C.).

Fraction M. 2:4-Di-O-methyl-D-galactose and 2-O-methyl-D-galactose.

Paper chromatography showed that this fraction was a mixture of 2:4-di-O-methyl-, and 2-O-methyl-D-galactose (in quantity). The colourless syrup crystallised and had m.p. 130°. Recrystallisation from glacial acetic acid yielded crystals m.p. 147°-149°C., not depressed by admixture with an authentic specimen of 2-O-methyl-D-galactose.

Fraction P. Free sugars and methylated uronic acid.

This fraction was obtained as a brown glassy solid. After two treatments with charcoal it was obtained as a colourless syrup. Barium ions were removed by shaking the aqueous solution with resin Amberlite IR120.

Paper chromatogram that was run in the solvent (e) showed a cherry spot (R_G 0.84) the colour and shape of which were similar

to those of a methylated uronic acid (56). Galactose and arabinose were also present.

Fraction F. Tri-O-methyl-D-galactose.

This fraction crystallised when it was inoculated with a crystal of tri-O-methyl-D-galactose from the undegraded polysaccharide.

Recrystallisation from acetone-ether-light petroleum gave crystals m.p. 53°-55°C. Further recrystallisation did not increase the melting point.

OMe = 37.8% (calculated $C_9H_{18}O_6 \cdot H_2O$, 38.8%).

Optical rotation.

The product was dried in vacuo at 60°C over phosphoric oxide for two hours,

$[\alpha]_D^{22}$ + 106° (constant), (c = 1.1 in water).

When the product was not dried, it showed an optical rotation $[\alpha]_D^{19}$ + 134° (5 min.), 122° (30 min.), 113° (1 hr.), 106° (2 hrs., constant) (c = 1.0 in water).

Preparation of anilide.

A sample (100 mg.) was heated with aniline (200 mg.) in alcohol (5 ml.) under reflux on the boiling water-bath (3 hrs.).

The bulk of the solvent was distilled off under reduced pressure and the residuum taken up in alcohol. After 24 hours at 0°C., crystals had deposited. They were filtered off and recrystallised from alcohol to yield the plate shaped 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine m.p. 164°C., not depressed by admixture with an authentic specimen. In admixture with an authentic specimen of 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine (m.p. 171°C.) the melting point was depressed to 144°C.

After the mother-liquor had been kept at 0°C. for several days a new batch of 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine was filtered off, m.p. 164°C. after recrystallisation from ethyl acetate.

The third batch of crystals was a mixture of plates and needles, probably 2:3:4-tri-O-methyl-, and 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine. According to Hirst and Jones (57) is the presence of a small quantity of either admixed with the other readily detected by microscopic examination, and a mixture of the two anilides shows a large depression in melting point.

The mixture of the two anilides after recrystallisation from alcohol had m.p. 143°C. It was not succeeded to separate them by flotation (57).

Reduction with Potassium Borohydride (72).

2:4-Di-O-methyl-D-galactose and the tri-O-methyl-D-galactose fractions from undegraded and degraded polysaccharides were reduced to the respective methylated galactitols by means of potassium borohydride. The sugar (200 mg.) was dissolved in water (10 ml.) and a solution of potassium borohydride (60 mg.) in water (5 ml.) added. After 14 hours the excess of potassium borohydride was destroyed by glacial acetic acid, and the solution was deionised by passing it through columns of the resins Amberlite IRA-400 and Zeo-Karb 225. The water was distilled off under reduced pressure to leave a crystalline residuum, yield about 50%.

The galactitols were recrystallised from alcohol-light petroleum to give white fibrous material.

2:4-di-O-methyl-D-galactitol, m.p. 133°-134°C.

$[\alpha]_D^{20}$ + 16° (c = 0.3 in water).

2:3:4-Tri-O-methyl-D-galactitol (in quantity) admixed with 2:4:6-tri-O-methyl-D-galactitol, m.p. 119°C. $[\alpha]_D^{20}$ + 6° (c = 0.8 in water).

The Estimation of Formaldehyde liberated during the Oxidation of the Methyl Ethers of Galactitol with Periodate (73).

Bell (71) has shown that some methylated sugars are oxidised slowly by periodate and the quantities of formaldehyde

are considerably below theoretical. In this work 2:4-di-O-methyl-D-galactitol was found to produce theoretical amounts of formaldehyde, though the oxidation was slower than it was for glucose.

The molecular proportions of 2:3:4-, and 2:4:6-tri-O-methyl-D-galactose in the tri-O-methyl-D-galactose fractions were estimated by means of the formaldehyde produced during the oxidation of the reduced sugars with periodate. The method of O'Dea and Gibbons (73) was used. As standard substance was used 2:4-di-O-methyl-D-galactitol assuming that this substance would be oxidised in the same manner as 2:3:4-tri-O-methyl-D-galactitol. The crude substances obtained after the reduction were not recrystallised before they were oxidised.

The methylated hexitols (5-15 mg.) after drying *in vacuo* at 60°C. (2 hours) were dissolved in water and diluted to 100 ml. in a standard flask. One volume of this solution was added to freshly prepared periodate - bicarbonate solution (1 vol.) and oxidised in the dark. At intervals a sample (1 ml.) was withdrawn, and pipetted into lead dithionate solution (10%;1 ml.) in a centrifuge tube. After mixing and centrifuging, a portion (1 ml.) of the supernatant liquid was withdrawn, placed in a second centrifuge tube, and chromotropic acid (9 ml.) was added. The mixed reagents were allowed to stand for 30 minutes; the lead sulphate was removed by centrifugation and the supernatant

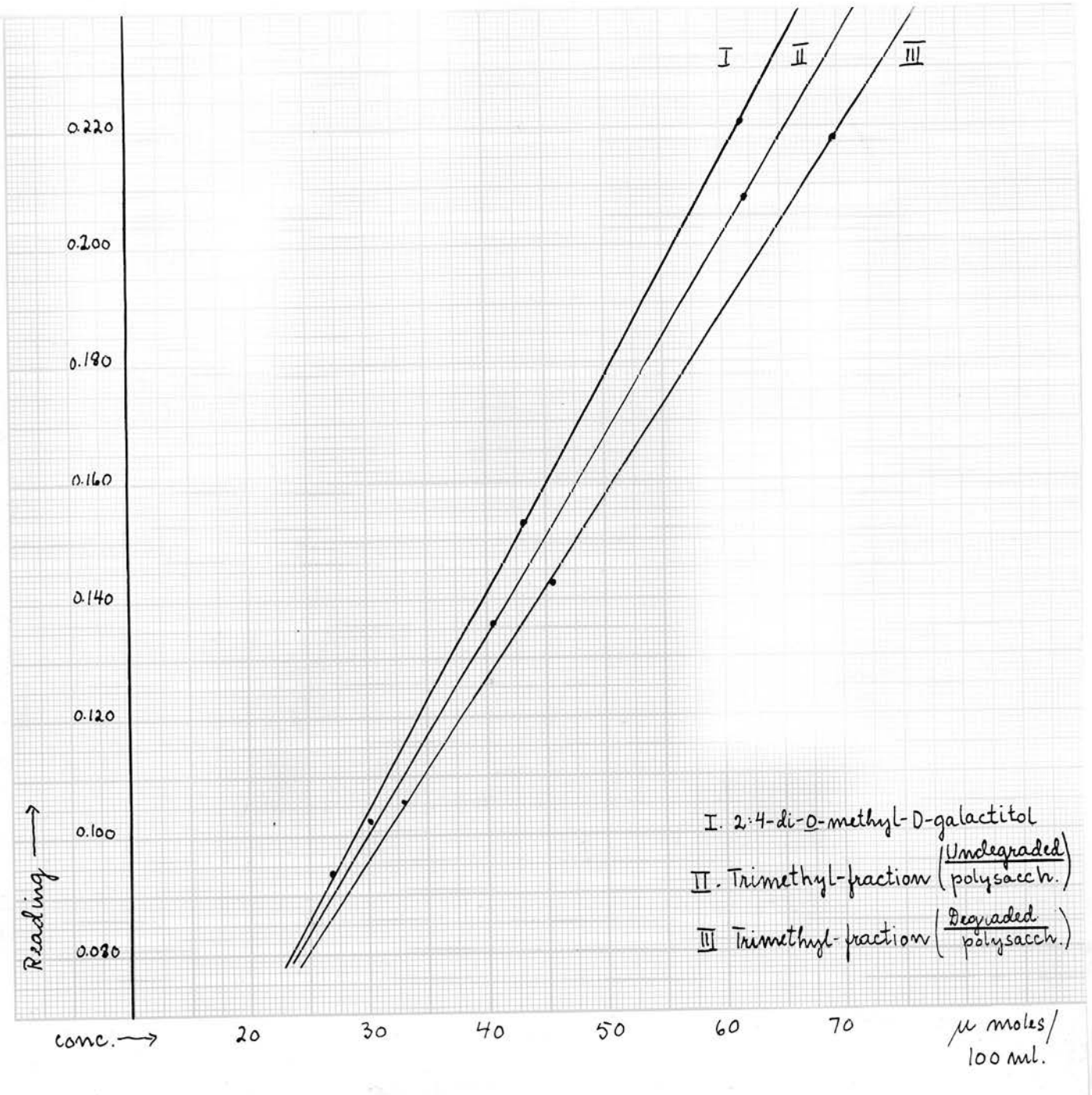
fluid transferred to a glass-stoppered tube and heated in the boiling water-bath for 40 minutes. The side of the tube was cooled by a coil condenser. All the operations were carried out away from direct light. A blank was run each time, only omitting the methylated hexitol. The absorption, using an Ilford spectrum yellow (606), was read on a Hilger photo-electric absorptiometer.

	mg. substance/ 100 ml.solution.	Reading (1 cm.cell)
di-methyl-galactitol	5.73	0.094
" " "	9.04	0.153
" " "	13.02	0.221
tri-methyl-galactitol	6.80	0.103
(undegraded)	9.10	0.136
" " "	13.84	0.208
tri-methyl-galactitol	7.47	0.106
(degraded)	10.18	0.143
" " "	15.52	0.218

The oxidation was complete after 2-4 days, depending on the amount of material to be oxidised.

The reading (corrected for blank) was plotted against the

concentration of the solution (μ moles/100 ml., w/v), and the molecular proportions of 2:4:6-tri-O-methyl-, and 2:3:4-tri-O-methyl-D-galactose read from the curves. The ratio was found to be approximately 1:16 for undegraded, and 1:8 for degraded polysaccharide.



DISCUSSION

DISCUSSION

The object of this research was to investigate ε-galactan from Larix decidua by means of methylation studies and periodate oxidation with the application of the modern technique of partition chromatography to the separation of the sugars and their derivatives.

Concurrently with the work on the original ε-galactan there was carried out an investigation of a degraded ε-galactan. The degraded polysaccharide was prepared by Dr. J.K.N. Jones, Bristol, by giving the original polysaccharide two treatments with boiling 0.01N-hydrochloric acid (1.5 hours). The degraded polysaccharide was recovered from the solution after neutralisation by precipitation with alcohol. Dr. Jones (10) has investigated the hydrolytic products which were left in solution. He found arabinose, galactose, one unidentified pentose containing oligosaccharide, one unidentified hexose containing disaccharide, and the disaccharide 3-O-β-L-arabopyranosyl-L-arabinose.

The undegraded and the degraded polysaccharides were pale yellow hygroscopic powders, readily soluble in water. They both had an optical rotation $[\alpha]_D$ + 19° (approximately) in water. The aqueous solutions were red-brown and dark which made it difficult to get distinct polarimeter readings except for very dilute solutions.

The polysaccharides were completely hydrolysed by N-sulphuric

acid at 100°C. The amounts of arabinose and galactose produced on hydrolysis were estimated by quantitative paper chromatography. The sugars were oxidised with sodium meta-periodate and the liberated formic acid titrated with 0.01N-sodium hydroxide. The undegraded polysaccharide contained 11.9% araban and 84.9% galactan which are in general agreement with results obtained by other workers (18,25,27,35). The degraded polysaccharide contained 8.0% araban and 88.3% galactan; similar results are obtained by Heidelberger (35,36).

On partial hydrolysis of the polysaccharide with 0.01N-sulphuric acid at 100°C., galactose and arabinose were found among the hydrolysis products. There were also some oligosaccharides, one of which had an R_G value on the paper chromatogram corresponding to that of 3-O-β-L-arabopyranosyl-L-arabinose. That this disaccharide did not arise by resynthesis was shown by hydrolysis of the polysaccharide with 0.5N-sulphuric acid at 100°C. After hydrolysis for one hour the disaccharide appeared on the paper chromatogram, but it disappeared on prolonged hydrolysis.

During the hydrolysis with 0.01N-sulphuric acid the degraded polysaccharide was recovered at intervals by precipitation in alcohol. The amount of arabinose and galactose was estimated by quantitative paper chromatography. After 16 hours hydrolysis the araban remaining in the polysaccharide was 1.2%. The backbone of the polysaccharide was evidently attacked during the hydrolysis

since galactose as well as arabinose was liberated. The weight of the degraded polysaccharide which was recovered at different stages decreased rapidly with each hydrolysis. When the araban content of the remaining polysaccharide had reached such a low value as 1.2%, only small quantities of the polysaccharide could be recovered by precipitation from the aqueous solution by the addition of alcohol.

Methylation studies.

The most important method in determining the constitution of a polysaccharide is the methylation and the examination of the cleavage products obtained on hydrolysis. The polysaccharides were completely methylated by three series of five methylations each with dimethyl sulphate and sodium hydroxide in an atmosphere of nitrogen. The purified methylated polysaccharides both contained OMe = 44.3% and had an optical rotation $[\alpha]_D$ - 49° (chloroform). The low optical rotation of the methylated product indicated that the galactose units were β-linked.

The araban (calculated as dimethyl araban) present in the methylated products was estimated by distillation with 12% hydrochloric acid and the furfural in the distillate precipitated with phloroglucinol. The methylated undegraded polysaccharide contained 5.2% dimethyl araban, and the methylated degraded polysaccharide contained 4.1% dimethyl araban. These results were in agreement with the amount of the dimethyl ethers of

arabinose obtained after the methylated polysaccharides had been hydrolysed and separated into their respective components.

An attempt was made to fractionate the methylated polysaccharides into fractions of different araban content by the solution method using chloroform and light petroleum. The precipitation method was also tried where the methylated product was precipitated from acetone solutions with light petroleum. The fractions achieved had similar araban content which showed that no significant fractionation had taken place. The same results have been reached in Japan by Tachi and Yamamori (27) when they investigated an arabogalactan from Japanese larch. Neither the two Japanese workers nor the writer managed to repeat the successful fractionation carried out by Campbell, Hirst and Jones (18) on the methylated galactan from European larch.

The completely methylated polysaccharides were hydrolysed first at room temperature with 2N-sulphuric acid. The solution was diluted with water, and the hydrolysis continued at 100°C. with N-sulphuric acid.

After complete hydrolysis the solution was neutralised with barium carbonate, filtered, and concentrated *in vacuo* to give a pale yellow syrup of the free methylated sugars.

Preliminary examination on the paper chromatogram of the mixture of methylated sugars showed that they had R_G values corresponding to the following sugars, tri-*O*-methyl-L-arabofuranose, tetra-*O*-methyl-D-galactose, di-*O*-methyl-L-arabofuranose,

tri-O-methyl-L-arabopyranose, tri-O-methyl-, di-O-methyl-, and mono-O-methyl-D-galactose, together with traces of unmethylated arabinose and galactose, and an unknown methylated uronic acid.

The hydrolysis products were separated on a column of powdered cellulose which provides the most convenient and rapid method of separating mixtures of monosaccharides or their methylated derivatives on a macro scale.

Since the preliminary examination by paper chromatography had shown that the differences in R_G values of the more methylated sugars were small, a complete separation of these constituents was not achieved.

The major part of the mixture consisted of 2:3:4:6-tetra-O-methyl-D-galactose, tri-O-methyl-D-galactose, and 2:4-di-O-methyl-D-galactose found in approximately equimolecular proportions for both polysaccharides. They were characterised as the respective anilides.

The tri-O-methyl-D-galactose fraction, however, presented a difficult separation problem. Galactose was the only free sugar which could be detected on the paper chromatogram after demethylation. Paper chromatography of the fraction indicated that it could be either 2:3:4-tri-O-methyl-D-galactose or 2:4:6-tri-O-methyl-D-galactose, or it might be a mixture of both because they have similar R_G values (solvent a)(52). The methoxyl content of the fraction (OMe = 38%) showed that the

monohydrate of the tri-O-methyl-D-galactose was present.

The low melting point (56°C.) which, however, was not altered by recrystallisation, indicated that this fraction was a mixture. The tri-O-methyl-D-galactose fractions from the undegraded and degraded polysaccharides had final optical rotations $[\alpha]_D$ + 109° and $[\alpha]_D$ + 106° (in water) respectively. The rotation value showed that the fractions could neither be pure 2:3:4-tri-O-methyl-D-galactose nor pure 2:4:6-tri-O-methyl-D-galactose while the following values are reported for these sugars:

2:3:4-tri-O-methyl-α-D-galactose monohydrate m.p. 75°-80°C., $[\alpha]_D$ + 152° → +114° (50,53),

2:4:6-tri-O-methyl-α-D-galactose m.p. 102°-105°C., $[\alpha]_D$ + 124° → + 90° (54).

Preparation of the anilide gave first pure 2:3:4-tri-O-methyl-N-phenyl-D-galactosylamine in good yield. The mother-liquor crystallised further to yield the plate shaped crystals of this anilide in admixture with some needles which were detected by microscopic examination. The needles were probably 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine (57). It was not possible to separate the two anilides, and the mixture of the two differently shaped crystals showed the same depression of melting point as a mixture did consisting of the authentic

specimens of 2:3:4- and 2:4:6-tri-O-methyl-N-phenyl-D-galactosylamine.

The value of the equilibrium rotation was lowest for the tri-O-methyl-D-galactose fraction from the degraded polysaccharide which indicated that this contained more 2:4:6-tri-O-methyl-D-galactose than the tri-O-methyl fraction from the original polysaccharide.

Standard solutions of the two methylated sugars were spotted on paper chromatograms and run in a number of different solvents, but it appeared that the two compounds had the same rate of movement and could not be separated by this method. Electrophoresis on paper was tried, but gave the same result as paper chromatography.

Unsubstituted sugars will on oxidation by periodate produce one mole of formaldehyde from the primary alcoholic group at carbon C6. When the hydroxyl group at carbon C6 is substituted the production of formaldehyde cannot take place. If 2:3:4-tri-O-methyl-D-galactose had produced theoretical amounts of formaldehyde during the oxidation by periodate an application of this method would make it possible to estimate the relative proportions of 2:3:4-tri-O-methyl- and 2:4:6-tri-O-methyl-D-galactose in a mixture of the two sugars. Bell (71) has shown, however, that some methylated sugars, among others are 2:4-di-O-methyl-D-galactose and 2:3:4-tri-O-methyl-D-glucose, are oxidised very slowly and produce less than theoretical quantities of

formaldehyde. In the present work the methylated sugars were accordingly reduced by potassium borohydride to the respective methylated hexitols in order to open the oxygen bridge in the sugar molecule. 2:4-Di-O-methyl-D-galactitol was found to produce theoretical amounts of formaldehyde on oxidation by periodate. This di-O-methyl-hexitol (which was available from 2:4-di-O-methyl-D-galactose from the methylated polysaccharide) was used as a standard substance assuming that it would be oxidised in the same manner as 2:3:4-tri-O-methyl-D-galactitol.

The molecular proportions of 2:4:6-tri-O-methyl- and 2:3:4-tri-O-methyl-D-galactose were found to be approximately 1:16 in the undegraded and 1:8 in the degraded polysaccharide.

Besides tetra-, tri-, and di-O-methyl-D-galactose there were small amounts of 2-O-methyl-D-galactose. This sugar was characterised by its rate on a paper chromatogram where it moved faster than ribose (solvent a). The identity was further confirmed by the melting point which showed no depression when mixed with an authentic specimen of 2-O-methyl-D-galactose. The undegraded polysaccharide contained more of this fraction than the degraded.

The arabinose constituent of the polysaccharide was found mainly as 2:5-di-O-methyl- and 2:3:4-tri-O-methyl-L-arabinose. There was also some 2:3:5-tri-O-methyl-L-arabinose present, but this fraction was too small to be examined and could only be

characterised on the paper chromatogram by its rate of movement and its grey colour after spraying with aniline oxalate. 2:5-Di-O-methyl-L-arabinose gave the same grey spot (pink in ultraviolet light) after spraying and it was easy to distinguish between this sugar and the other di-O-methyl ethers of arabinose. 3:5-Di-O-methyl-L-arabinose had the same rate of movement but gave a brown colour (yellow in ultraviolet light) with aniline oxalate. The di-O-methyl-L-arabopyranoses travelled more slowly and gave a pink colour with aniline oxalate. 2:3:4-Tri-O-methyl-L-arabinose gave also a pink colour, and the rate of movement of this sugar was more slowly than that of 2:5-di-O-methyl-L-arabinose in most solvents except for benzene-ethanol-water.

The fraction containing 2:5-di-O-methyl- and 2:3:4-tri-O-methyl-L-arabinose was obtained as a syrupy mixture of the two sugars. They were separated on thick paper chromatograms, but a complete separation of the two sugars was not achieved due to streaking.

The optical rotation of the tri-O-methyl-L-arabinose fraction was $[\alpha]_D$ + 120° (water). The arabonolactone obtained after the sugar had been oxidised with bromine was converted into the phenylhydrazide.

The di-O-methyl-L-arabinose fraction had a negative solution. The fraction obtained from the degraded polysaccharide was probably the most pure and had $[\alpha]_D$ -9° (water). The sugar was oxidised with bromine and the 2:5-di-O-methyl-L-arabonamide prepared.

2:3:4-Tri-O-methyl-L-arabinose and 2:5-di-O-methyl-L-arabinose were found in the molecular ratio 1:1.5 for undegraded and 1:1 for degraded polysaccharide.

When Jones (10) isolated the disaccharide 3-O-β-L-arabopyranosyl-L-arabinose after partial hydrolysis of larch galactan he obtained 2:3:4-tri-O-methyl- and 2:4-di-O-methyl-L-arabinose after methylation and hydrolysis of the disaccharide. He suggested, however, that it existed in the polysaccharide as 3-O-β-L-arabopyranosyl-L-arabofuranose, and the result from the present work confirmed that; there might even in some cases have been two arabofuranose units attached to one arabopyranose unit, Ap1 - 3Af1 - 3Af1 - Ga - (Ap = arabopyranose residue, Af = arabofuranose residue, Ga = galactopyranose residue), because the undegraded polysaccharide contained more of di-O-methyl-L-arabinose than of tri-O-methyl-L-arabinose.

The last fraction which was collected from the cellulose column consisted of a mixture of free sugars and a methylated uronic acid. These constituents were only examined on the paper chromatogram, and because of the small quantities it is doubtful whether they are of structural significance.

The quantities of methylated sugars which were isolated

from the cellulose column were as follows,

	Undegraded polysacch.	Degraded polysacch.
Tetramethyl-galactose	5.0 moles^{-3}	5.7 moles^{-3}
Trimethyl-galactose	4.4 "	4.9 "
Dimethyl-galactose	5.8 "	5.2 "
Monomethyl-galactose	1.3 "	1.1 "
Dimethyl-arabinose	0.8 "	0.5 "
Trimethyl-arabinose	0.5 "	0.5 "
Methylated uronic acid	ca. 2%	ca. 2%

Periodate oxidation.

Sugar derivatives containing hydroxyl groups on each of three adjacent carbon atoms are oxidised by salts of periodic acid with the formation of one mole of formic acid and the consumption of two moles of periodate. Sugars containing hydroxyl groups on each of two adjacent carbon atoms will not produce any formic acid during the oxidation but will consume one mole of periodate (58). By measuring the amount of formic acid liberated and the consumption of periodate during the

oxidation of the polysaccharide by the periodate ion, one will obtain valuable information about the fine structure of the polysaccharide.

Undegraded and degraded ε-galactan were oxidised by the periodate ion, and the periodate consumption and the formic acid liberated were estimated by titration. After 25 hours there were released 3 moles of formic acid and consumed 7.7 moles of periodate per repeating unit of mol. wt. 1104 for undegraded and mol. wt. 1060 for degraded polysaccharide. After this time the amount of formic acid liberated increased slowly and was constant after 360 hours, when 4 moles of formic acid had been liberated. The amount of periodate consumed was 8 moles for undegraded and 8.5 moles for degraded polysaccharide.

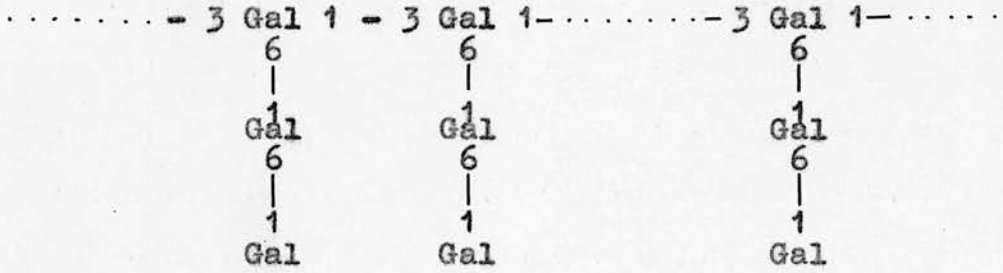
Brown, Dunstan, et al. (34) reported they had found three end groups per repeating unit of mol. wt. 1104.

The fourth mole of formic acid liberated might be due to over-oxidation, but taken in conjunction with the methylation studies where there were found about equimolecular proportions of 2:3:4:6-tetra-O-methyl-, 2:3:4-tri-O-methyl-, and 2:4-di-O-methyl-D-galactose, the results obtained after 360 hours oxidation seem to be most correct.

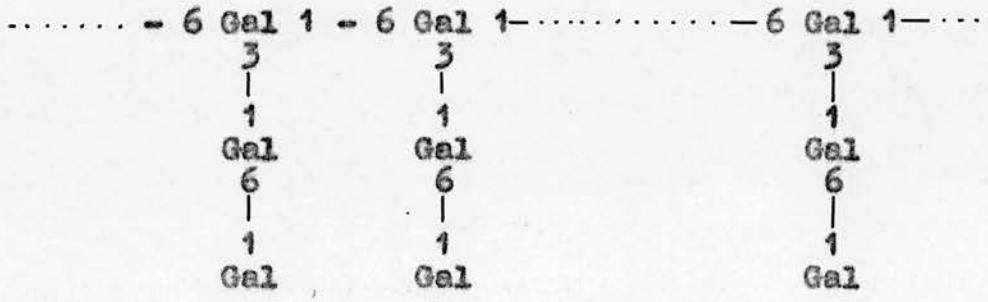
After the oxidised material had been dialysed and hydrolysed, galactose and arabinose were found on the paper chromatogram. The fact that arabinose was present in the

oxidised material was in agreement with the result from the methylation studies. It appeared from these observations that arabinose cannot be present entirely as end groups in the polysaccharide as has been suggested by White (32) for the arabogalactan from Western larch.

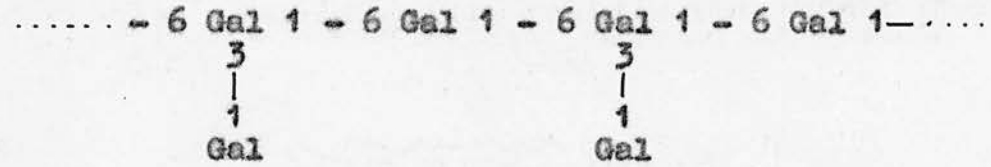
The methylation studies revealed the fact that the main portion of the polysaccharide was built up from galactose units giving 2:3:4:6-tetra-O-methyl-, 2:3:4-tri-O-methyl-, and 2:4-di-O-methyl-D-galactose in about equimolecular proportions. This indicated that the backbone of the polysaccharide might consist of galactose units linked either through carbon C1-C3, or through carbon C1-C6, or through carbon C1-C6 and through carbon C1-C3. Some of the simplest possibilities are shown in the following formulae,



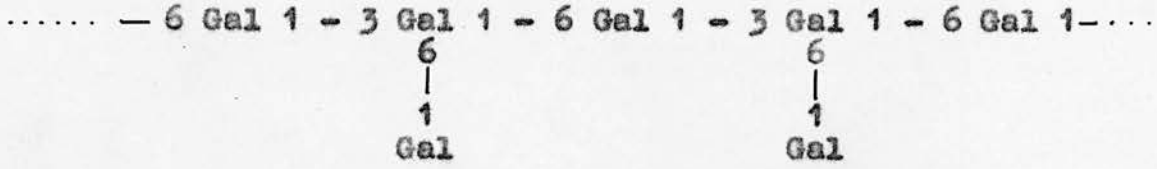
I



II



III



IV

Gal = β-D-galactopyranose residue.

Periodate oxidations of the polysaccharides (I) and (II) would leave the main chain [- 3 Gal 1 - 3 Gal 1 -] or [- 6 Gal 1 - 6 Gal 1 -] unattacked, while in the polysaccharides (III) and (IV) the side chains as well as the sugar residues in the main chain would be oxidised by the periodate ion.

In order to try to get some further insight into this problem the periodate oxidised polysaccharide was degraded by the method of Barry (63,66).

Barry (61) found that the glycosidic linkage in periodate oxidised starch was readily opened by treatment with phenylhydrazine acetate under mild conditions. Such 1:4 linked periodate oxidised polysaccharides where the carbon chain in each sugar unit is ruptured between carbon atoms C2 and C3 are completely degraded by Barry's method giving the osazones of glyoxal and erythrose. The method may also be used to bring about a regulated degradation of 1:3 linked polysaccharides where the oxidised terminal units are completely removed by phenylhydrazine.

Barry (62) has also shown that periodate oxidised polysaccharides form polymeric condensation products with phenylhydrazine, isonicotinoylhydrazide or semicarbazide.

Many polysaccharides contain both sugar residues which are oxidised by periodate and residues which do not contain α-glycol groups and, therefore, are not oxidised. Since the latter are usually in the inner part of the molecule, the application of

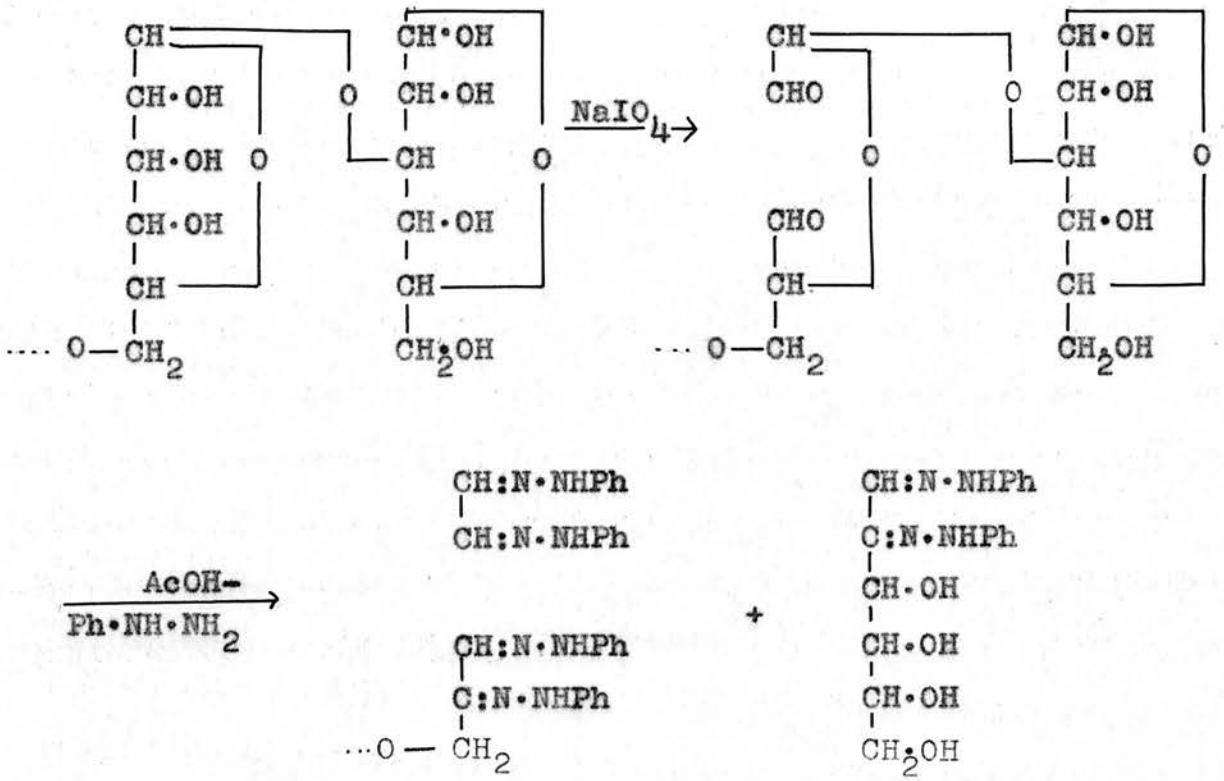
the Barry reaction to the periodate oxidised materials should leave the unoxidised portions as polysaccharide in many cases.

Periodate oxidised larch galactan was degraded by phenylhydrazine acetate followed by periodate oxidation, another degradation, and finally again oxidised by periodate. The products were isolated at different stages, hydrolysed, and examined for galactose and arabinose. Galactose and arabinose were present; the amount of arabinose decreased after each oxidation.

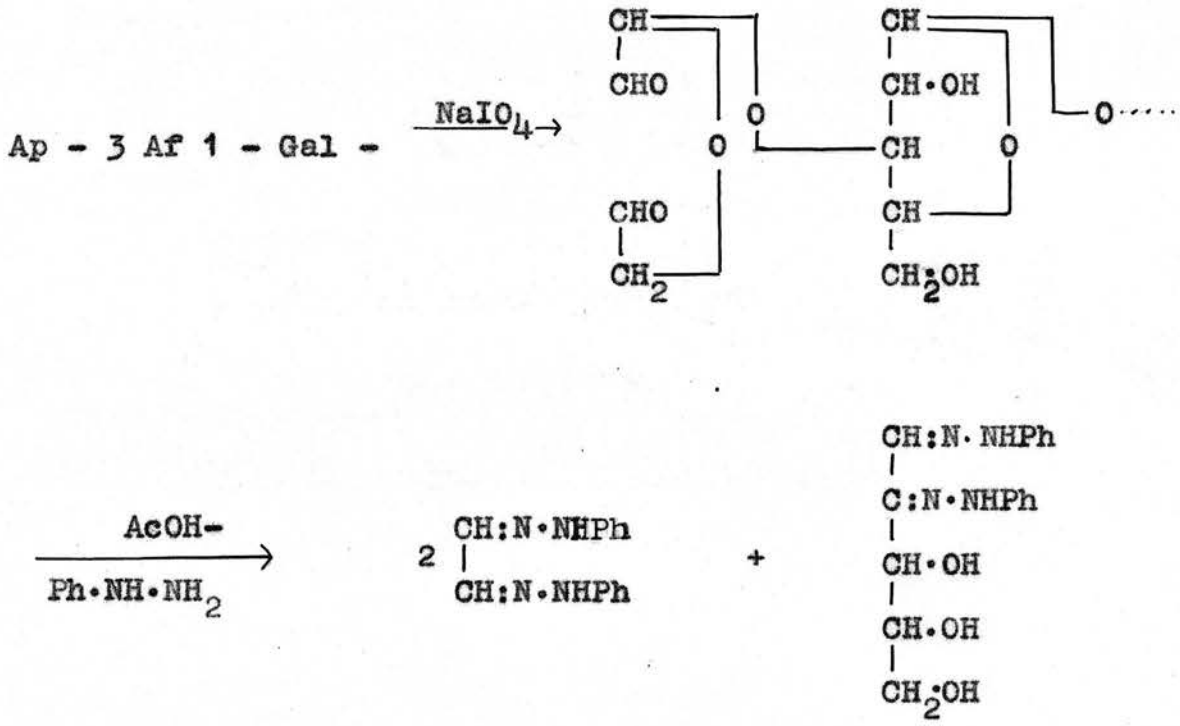
In another experiment the ether soluble fragments obtained after the first Barry degradation were separated on an alumina column and examined by means of circular paper chromatography. The osazones of glyoxal and glycerose were obtained crystalline. The osazones of arabinose and galactose were found on the circular paper chromatogram. The water soluble part which consisted of the remainder of the polysaccharide was partially hydrolysed with 0.5N- sulphuric acid. Examination on the paper chromatogram showed that arabinose and galactose were among the hydrolysis products together with a hexose containing disaccharide. This might be an indication that the backbone of the polysaccharide has a structure that was indicated by the formulae (I) and (II). It has been suggested (1a) that the situation here is not unlike that in the xylan group where larger or smaller proportions of L-arabinose residues may be attached to a basal structure composed of xylose residues (see p.4).

But the structure of larch galactan is probably more complicated since 2:3:5-tri-O-methyl-, 2:5-di-O-methyl-, and 2:3:4-tri-O-methyl-L-arabinose as well as 2-O-methyl-D-galactose were found among the hydrolytic products of the methylated polysaccharide. The larger amount of 2:4:6-tri-O-methyl-D-galactose in the degraded polysaccharide indicated that a galactose residue linked through C3 had also had a sugar residue attached to carbon C6, but the latter was removed during the partial hydrolysis.

Another evidence for the presence of 1:3 linkages in the main chain of the polysaccharide is the presence of galactosazone after the Barry degradation. This might have been produced by a reducing end group linked through carbon C3 to another residue which was oxidised by periodate.



The formation of the osazone of arabinose was probably caused by hydrolysis with acetic acid. Furanose units are easily hydrolysed by carboxylic acids at 100°C.



About one-third of the D-galactose residues were triply linked through carbon C1, C3, and C6, and one-third were double linked through carbon C1 and C6 (in a few cases through C1 and C3 we know with certainty from the presence of 2:4:6-tri-O-methyl-D-galactose). It appears that D-galactose favours the 1:3 and 1:6 type of linkage which is typical of its occurrence in several plant gums (12).

As for the arabinose units it appeared that they must be attached to galactose residues in the parent polysaccharide. The relative proportions of 2:3:4-tri-O-methyl-L-arabinose and 2:5-di-O-methyl-L-arabinose together with the absence of mono-O-methyl-L-arabinose made it certain that there cannot exist a pure araban. The work by Heidelberger (35,36) showed the same when no fractionation of the polysaccharide took place on the precipitation of antibody in Type XIV antipneumococcal horse sera. It is possible that the situation here is not unlike that in the xylan group. The high proportion of araban in wheat-flour araboxylan could for example not be altered by fractionation and structural investigations showed that all arabinose residues were attached as end groups to the xylose residues in the parent polysaccharide. On the other hand araboxylan from Esparto grass can be fractionated into one pure xylan and one araboxylan.

As in the case of larch galactan it seems that we have to deal with a large group of polysaccharides with very similar main-chain structures but with very varied proportions of side chains. It appears from the present results that arabinose can exist in the polysaccharide either as terminal single arabofuranose residues, or as terminal arabopyranose units linked to one or two arabofuranose residues. It is not possible to formulate a unique structure for ε-galactan, but the large amount of end group showed that the molecule must be of the highly branched type.

From the present results it is difficult to distinguish between structural formulae such as (I) and (II), and the main chain may even consist of alternating 1:3 and 1:6 linkages as in formula (IV) but with a side chain attached to each galactose unit. But a closer investigation of the carbohydrate material left from the Barry degradation may help to clear this up.

SUMMARY

1. The water soluble polysaccharide ε-galactan was prepared at Forest Products Research Laboratory, Princes Risborough, Bucks., by water extraction of sawdust from European larch. The polysaccharide was a pale yellow hygroscopic powder with optical rotation in water $[\alpha]_D$+19° (approximately). ε-Galactan contained 84.9% galactan and 11.9% araban estimated after complete hydrolysis.

2. On mild hydrolysis of ε-galactan there was obtained a degraded polysaccharide with similar physical properties to those of the original sample. The degraded larch galactan was also a pale yellow hygroscopic powder, easily soluble in water with an optical rotation $[\alpha]_D$+19°. It contained 88.3% galactan and 8.0% araban.

3. Attempts to prepare a galactan free from arabinose failed because galactose and hexose containing polysaccharides were liberated during the hydrolysis.

4. On treatment with sodium meta-periodate larch galactan consumed 8 moles of periodate and liberated 4 moles of formic acid per repeating unit of mol. wt. 1104. Similarly degraded larch galactan consumed 8 moles of periodate and liberated 4 moles of formic acid per repeating unit of mol. wt. 1060. Hydrolysis of the periodate oxidised polysaccharides showed that both contained galactose and arabinose.

5. The periodate oxidised larch galactan was degraded with phenylhydrazine in acetic acid by the method of Barry. Among the degradation products were detected glyoxalbisphenylhydrazone, glycerosazone, and traces of the osazones of arabinose and galactose. The remaining carbohydrate material gave arabinose, galactose, and a hexose containing disaccharide on partial hydrolysis.

6. The polysaccharides were converted into the respective methyl ethers. The methylated products were subjected to fractionation but it was not possible to obtain distinct molecular species by this method.

7. Methylated ε-galactan gave on hydrolysis 2:3:4:6-tetra-O-methyl-D-galactose (5 moles), tri-O-methyl-D-galactose (4 moles), 2:4-di-O-methyl-D-galactose (6 moles), 2-O-methyl-D-galactose (1 mole), 2:3:5-tri-O-methyl-L-arabinose (trace), 2:5-di-O-methyl-L-arabinose (0.8 moles), 2:3:4-tri-O-methyl-L-arabinose (0.5 moles), methylated uronic acid (ca. 2%).

Methylated degraded ε-galactan gave on hydrolysis 2:3:4:6-tetra-O-methyl-D-galactose (6 moles), tri-O-methyl-D-galactose (5 moles), 2:4-di-O-methyl-D-galactose (5 moles), 2-O-methyl-D-galactose (1 mole), 2:3:5-tri-O-methyl-L-arabinose (trace), 2:5-di-O-methyl-L-arabinose (0.5 moles), 2:3:4-tri-O-methyl-L-arabinose(0.5 moles), uronic acid (ca. 2%).

8. Evidence was found that the tri-O-methyl-D-galactose fraction was a mixture of 2:4:6- and 2:3:4-tri-O-methyl-D-galactose, the latter being preponderating. A method was worked out to estimate the proportions of the two methyl ethers in mixture.

9. It is concluded from these results that ε-galactan from European larch is a highly branched arabogalactan, and the possible molecular structures have been discussed.

BIBLIOGRAPHY

1a. Hirst, J.C.S., 1955, 2976.

1. Chanda, Hirst, Jones, Percival, J.C.S., 1950, 1289.

2. Perlin, Cereal Chem., 1951, 28, 370, 382.

3. Aspinall, Hirst, Moody, Percival, J.C.S., 1953, 1631.

4. Hirst, Jones, J.C.S., 1947, 1222.

4a. Andrews, Hough, Jones, J.C.S., 1954, 806.

5. Hirst, Jones, Walder, J.C.S., 1947, 1225.

6. Stockmann, Hägglund, Svensk Papperstidn., 1948, 51, 269.

7. Schorger, Smith, Ind. Eng. Chem., 1916, 8, 494.

8. Foremann, Englis, Ind. Eng. Chem., 1931, 23, 415.

9. Wadmann, Anderson, Hassid, J.A.C.S., 1954, 76, 4097.

9a. Turton, Bebbington, Dixon, Pacsu, J.A.C.S., 1955, 77, 2565.

9b. Ball, Jones, Nicholson, Hall, Division Carbohydrate Chemistry, J.A.C.S., Sept. 1955.

10. Jones, J.C.S., 1953, 1672.

11. Jones, J.C.S., 1954, 4134.

12. Andrews, Ball, Jones, J.C.S., 1953, 4090.

13. Jones, J.C.S., 1939, 558.

14. Jones, J.C.S., 1950, 534.

15. Jones, J.C.S., 1947, 1055.

15a. White, J.A.C.S., 1953, 75, 257.

16. Hirst, J.C.S., 1942, 70.

17. White, J.A.C.S., 1941, 63, 2871.

18. Campbell, Hirst, Jones, J.C.S., 1948, 776.

19. Whistler, Smart, Polysaccharide Chemistry, New York 1953.

20. Müntz, Compt. rend., 94, 454.

21. Steiger, Ber., 1886, 19, 827.

22. Lippmann, Ber., 1887, 20, 1001.

23. Bauer, Journ. prakt. Chem., 30, II, 283.

24. Trimble, Amer. J. Pharm., 1898, 70, 152.

25. Wise, Peterson, Ind. Eng. Chem., 1930, 22, 362.

26. Wise, Unkauf, Cellulosechemie, 1933, 14, 20.

27. Tachi, Yamamori, Bul. Agric. Chem. Soc., Japan, 1953, 27, 139.

27a. Nikitin, Soloviev, J. Appl. Chem., (U.S.S.R.), 1935, 8, 1016, Amer. Chem. Abstr., 1936, 30, 5563.

28. Zherebov, Gordon, Komarovskii, Trans. Central Inst. Sci. Research Forest Chem., (U.S.S.R.), 1933, 2,52, Amer. Chem. Abstr., 1934, 28, 4265.

29. Wise, Hamer, Peterson, Ind. Eng. Chem., 1933, 25, 184.

30. T. Ito, Cellulose Ind., (Tokyo), 1937, 13, 4. Amer. Chem. Abstr., 1937, 31, 6872.

31. Peterson, Barry, Unkauf, Wise, J.A.C.S., 1940, 62, 2361.

32. White, J.A.C.S., 1941, 63, 2871, 1942, 64, 302, 1507, 2838.

33. Campbell, Hirst, Jones, Nature, 1941, 147, 25.

34. Brown, Dunstan, Halsall, Hirst, Jones, Nature, 1945, 156, 785.

35. Heidelberger, J.A.C.S., 1955, 77, 4308.

36. Heidelberger, Private communication.

37. Husemann, Journ. prakt. Chem., 1940, 155, 13.

38. Owens, J.A.C.S., 1940, 62, 930.

39. Mosimann, Svedberg, Kolloid. Z., 1942, 100, 1.

40. Gerd L. Borgin, J.A.C.S., 1949, 71, 2247.

41. Flood, Hirst, Jones, Nature, 1947, 160, 86. J.C.S., 1948,1679.

42. Hirst, Jones, J.C.S., 1949, 1659.

43. Haworth, J.C.S., 1915, 107, 8.

44. Purdie, Irvine, J.C.S., 1903, 1021.

45. Menzies, J.C.S., 1926, 937.
Arni, Percival, ibid., 1951, 1822.

46. Bott, Hirst, J.C.S., 1932, 2621.

47. Charles Dorée, The Methods of Cellulose Chemistry, London 1947.

48. McCreath, Smith, J.C.S., 1939, 387.

49. Hough, Jones, Wadmann, Nature, 1948, 162, 448, J.C.S., 1949, 2511.

50. Smith, J.C.S., 1939, 1724.

51. Hough, Jones, Wadmann, J.C.S., 1950, 1702.

52. Hirst, Jones, Discussions Faraday Soc., 1949, 7, 268.

53. Laidlaw, Wylam, J.C.S., 1953, 567.

54. Bell, Williamson, J.C.S., 1938, 1196.

55. Lindberg, Wickberg, Acta Chem. Scand., 1954, 8, 569.

56. Eddington, Ph. D. Thesis, Edinburgh University, 1954.

57. Hirst, Jones, J.C.S., 1939, 1486.

58. Halsall, Hirst, Jones, J.C.S., 1947, 1399, 1427.

59. Barnebey, J.A.C.S., 1916, 38, 330.

60. Lythgoe, Todd, J.C.S., 1944, 592.

61. Barry, Nature, 1943, 152, 537.

62. Barry, Mitchell, J.C.S., 1953, 3610.

63. O'Colla, Proc. Royal Irish Acad., 1953, 55, Section B, 165.

64. Autenrieth, Ber., 1901, 34, 173.

65. Barry, McCormick, Mitchell, J.C.S., 1954, 3692.

66. Barry, Mitchell, J.C.S., 1954, 4020.

67. Rutter, Nature, 1948, 161, 435.

68. Giri, Nature, 1952, 169, 923, 1954, 173, 1194.

69. Hudson, J.A.C.S., 1925, 47, 265.

70. Hirst, Robertson, J.C.S., 1925, 358.

71. Bell, J.C.S., 1948, 992.

72. Abdel-Akher, Hamilton, Smith, J.A.C.S., 1951, 73, 4691.

73. O'Dea, Gibbons, Biochem. J., 1953, 55, 580.

74. Smith, J.C.S., 1939, 744.

ACKNOWLEDGMENTS.

The author wishes to record her gratitude to Professor E. L. Hirst, F.R.S., and Dr. G.O. Aspinall for their advice and encouragement throughout the course of this work. Thanks are also expressed to The University of Oslo and Pasteurlegatet for grants, and especially to Norges Almenvitenskapelige Forskningsråd (The Norwegian Council for Science and the Humanities) for a maintenance grant which enabled the author to carry out this work.